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Implementation Plan for Safe Pesticides/Safe Products Research

National Health and Environmental Effects Research Laboratory
Office of Research and Development

December, 2005

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Executive Summary

This document describes the implementation plan (the Plan) for research within the National Health and Environmental Effects Research Laboratory (NHEERL) under the Safe Pesticides/Safe Products (SP^2) Multiyear Plan to support the Office of Pollution Prevention, Pesticides and Toxic Substances (OPPTS) for fiscal years 2005 - 2010 and beyond.

ORD has developed a multi-year research plan to address OPPTS' needs for research. The NHEERL SP^2 research program described in the Plan contains four program areas targeted to OPPTS' major needs, focused respectively on:

a) prioritizing and ranking chemicals for testing and enhancing the interpretation of test data;
b) spatially explicit, geographically based probabilistic ecological risk assessment;
c) biotechnology; and
d) novel and newly discovered hazards

The document describes each of the projects proposed under these program areas, including a summary of the issue, state-of-the-science and research needs, along with the projected activities, resource requirements, critical path and key products.

The projects were developed with recognition that a key measure of success is the effectiveness with which critical information developed by NHEERL is used by our research partner, i.e., OPPTS, to make their program more effective and the make communities cleaner.

Foreword

The National Health and Environmental Effects Research Laboratory (NHEERL), as part of the Environmental Protection Agency's Office of Research and Development (ORD), serves as EPA's focal point for scientific research on the effects of contaminants and environmental stressors for both human health and ecosystem integrity. NHEERL's research helps the Agency identify and understand the processes that affect our health and environment, thereby aiding in evaluation of the risks that pollution poses to humans and ecosystems. The research is intended to address key Agency problems in a timely and responsive manner. In this context, NHEERL develops research implementation plans to achieve the following objectives:

a) Optimizing responsiveness of research activities to Agency needs,
b) Sharpening the focus of research programs where needed,
c) Providing a forum for engagement of scientific staff on issues and approaches,
d) Focusing on multi-year planning explicitly linked to Agency performance goals, and
e) Providing a mechanism for prioritizing research.

NHEERL's approach builds on the ORD planning process that identifies and prioritizes research needs. ORD's research portfolio includes both core and problem-driven program areas. Currently, ORD has problem-driven research programs for air, water, waste, and pesticides and toxic substances, each of which addresses key problems faced by the respective regulatory program

This NHEERL implementation plan identifies the scientific problems and research that will be conducted by NHEERL concerning Safe Pesticides/Safe Products and the key problem areas for EPA's Office of Pollution Prevention, Pesticides and Toxics (OPPTS). The goal of NHEERL's research in this area is to help ORD address the 4 major problem areas facing OPPTS that are not addressed in the other ORD Multiyear Plans, specifically:

a) prioritizing and ranking chemicals for testing and enhancing the interpretation of test data;
b) spatially explicit, geographically based probabilistic ecological risk assessment;
c) biotechnology; and
d) novel and newly discovered hazards.

This document was developed by representatives from NHEERL research divisions, with significant engagement and peer review from OPPTS and other ORD laboratories and centers. This draft document has not yet been reviewed by scientists external to the Agency. It is intended to reflect research that will be conducted over the next several years. As progress is made in achieving the goals outlined in this document, it will be updated to address new and remaining challenges.

Harold Zenick, Acting Director
National Health and Environmental Effects Research Laboratory

Peer Reviewers of NHEERL's Safe Pesticides/Safe Products Implementation Plan

TO BE DETERMINED

Acronyms Used

ACE	Acute-to-Chronic Estimation Software
ALSase	Acetolactate Synthase
APM	Annual Performance Measure
ASTER	ASsessment Tools for Ecological Risk
BTB	Blood-Testis Barrier
CDC	Centers for Disease Control
CLO	Cornell Laboratory of Ornithology
CNS	Central Nervous System
CT	Computational Toxicology
CTISC	Computational Toxicology Implementation Steering Committee
CWA	Clean Water Act
DNT	Developmental Neurotoxicology
DTB	Developmental Toxicology Bioassay
DTH	Delayed Type of Hypersensitivity
ECF	Electrochemical Fluorination
ECOFRAM	Ecological Committee on FIFRA Risk Assessment Methods
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EFED	Environmental Fate and Effects Division
ELISA	Enzyme-Linked Immunosorbant Assay
EMAP	Environmental Monitoring and Assessment Program
ER	Estrogen Receptor
ERA	Ecological Risk Assessment
ERASC	Ecological Risk Assessment Support Center
ESRI	Environmental Systems Research Institute
ETR	Electron Transport Rate
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
GIS	Geographical Information Systems
GM	Genetically Modified
GMO	Genetically Modified Organism
GUI	Graphical User Interface
HPG	Hypothalamic-Pituitary-Gonadal
HPLC	High Performance Liquid Chromatography
HPV	High Production Volume
HTPS	High Throughput System
ICE	Interspecies Correlation Estimation
LMC	Laboratory of Mathematical Chemistry
LOEL	Lowest Observed Effect Level
LTG	Long-Term Goal
LTP	Long-Term Potentiation
MMI	Methimazole
MOA	Mode Of Action
MYIP	Multi-Year Implementation Plan (NHEERL)
MYP	Multi-Year Plan (ORD)
NCDC	National Climate Data Center

NCEA	National Center for Environmental Assessment
NCFAP	National Center for Agricultural Policy
NERL	National Exposure Research Laboratory
NHEERL	National Health and Environmental Effects Research Laboratory
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institute of Health
NOAA	National Oceanic and Atmospheric Administration
NOEL	No Observed Effect Level
NRC	National Research Council
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
OPP	Office of Pesticide Programs
OPPT	Office of Pollution Prevention and Toxics
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
ORD	Office of Research and Development
PAM	Pulse Amplitude Modulated
PATCH	Program to Assist in Tracking Critical Habitat
PBDE	Polybrominated Diphenyl Ethers
PBTK	Physiologically Based Toxicokinetic
PCB	Polychlorinated Biphenyl
PCR	Polymerase Chain Reaction
PEA	Plant Efficiency Analyzer
PFAA	Perfluoroalkyl Acids
PFDA	Perfluorodecanoic Acid
PFHS	Perfluorohexane Sulfonate
PFNA	Perfluorononanoic Acid
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonate
PI	Photosynthesis-Irradiance
PIP	Plant Incorporated Protectant
PK	Pharmacokinetic
PMN	Pre-Manufacturing and Notification
PPAR-	Peroxisome Proliferator-Activated Receptor
PTU	Propylthiouracil
QSAR	Quantitative structure-activity relationship
RCRA	Resource Conservation and Recovery Act
SDWA	Safe Drinking Water Act
SP2	Safe Pesticides, Safe Products Multi-Year Implementation Plan
SSD	Species Sensitivity Distribution
SU	Sulfonylureas
TSCA	Toxic Substances Control Act
USDA	United States Department of Agriculture
WRS	NHEERL Wildlife Research Strategy

Introduction

Purpose and Scope

This document, the *NHEERL Implementation Plan for Safe Pesticides/Safe Products Research*, describes the research that the National Health and Environmental Effects Research Laboratory (NHEERL) intends to perform in support of the Office of Research and Development's (ORD's) multi-year research plan (MYP) for pesticides and other commercial chemicals. ORD uses multi-year planning to chart the direction of ORD's research programs in selected topic areas for time periods extending up to five to ten years.

To guide the implementation of strategic directions identified in the ORD MYPs, NHEERL develops multi-year implementation plans (MYIPs) for its major research programs. These plans are intended to ensure that NHEERL research addresses the key mission-related scientific issues that are raised in the ORD MYP in a way that maximizes benefit to the programmatic partner, utilizes fully NHEERL capability, and integrates effectively with related ORD research programs.

ORD Safe Pesticide/Safe Products Long-Term Goals

LTG 1: To provide OPPTS with predictive tools for prioritization of testing requirements & enhanced interpretation of hazard identification & dose-response information

LTG 2: To create the scientific foundation for OPPTS probabilistic risk assessment methods to protect natural populations of birds, fish, & other wildlife

LTG 3: To provide OPPTS with the scientific underpinnings for guidance to prevent or reduce risks of human environments with communities, homes, & workplaces [Note: this LTG needs refinement as it was designed to meet the safe community theme of the original multiyear plan]

LTG 4: To provide OPPTS with strategic scientific information & advice concerning novel or newly discovered potential hazards

Problem

The ability of the Office of Pesticide Programs (OPP) to require extensive testing for conventional pesticides make it data rich. Yet the program faces significant problems. Current regulations require costly testing, use of large numbers of animals, and provide data that is often less than optimal. OPP needs help to reduce the testing costs to industry, to reduce the volume of data that must be analyzed by OPP staff, and to reduce the number of animals used to register a pesticide. In other words, OPP requires a more efficient and effective testing process. NHEERL can help by developing tiered testing approaches. These approaches will require NHEERL to develop scientific bases for policies on decision criteria regarding the sufficiency of negative lower tier data to conclude that the agent does not pose an unreasonable risk and that further testing is not required.

By contrast, The Office of Pollution Prevention and Toxics (OPPT) does not have the legislative authority to require extensive testing. Their biggest need is the development of high throughput *in vitro* and Quantitative Structure Activity Relationships (QSAR) testing methods to evaluate the risks of substances for which there may be a significant new use or for which there is concern about widespread exposure with significant potential to cause adverse effects. When addressing substances other that conventional pesticides (e.g., anti-microbials and inerts), OPP has needs similar to OPPT, because it is not practical to test these products in as much depth as the conventionals for a variety of reasons. OPP needs methods to prioritize which chemicals should be tested first and to decide what tests should be conducted to assess the risk posed by exposure to these agents. NHEERL can help all of OPPTS through the development of QSAR to help prioritize

chemicals for testing requirements and through the development of high throughput testing to screen the large numbers of chemicals that need to be assessed.

One of the biggest challenges facing OPP is the need to more explicitly evaluate ecological risk of pesticides across all tiers of ecological risk assessment. Their needs range from screening tier tools to broadly assess the potential for ecological risk to higher tier tools to realistically evaluate specific risk scenarios. The major needs include: species-to-species extrapolations to enable a realistic use of information collected from one species to estimate the risks faced by another species; dose-response information to understand the response of key vital species to the pesticide of concern; population models to integrate dose-response information and project population impacts; understanding the impact of habitat alteration in conjunction with the pesticide exposure on individual- and population-risk; spatially explicit modeling tools to evaluate risks to populations across an agricultural landscape; and, the ability to assess the risks that pesticide use may have on endangered species.

Finding ways to address OPPTS' prioritization and efficiency needs for health effects data and finding ways to develop spatially explicit, geographically based ecological risk approaches are exciting and challenging scientific problems. Across the entire Agency, EPA needs to pull together all available information about production, release, exposure, and effects into a picture of risk. The many unknowns create exciting research opportunities. We believe that the research questions facing EPA will become more tractable as a result of new technologies coupled with rapidly advancing computer capabilities. However, these new approaches by themselves will not meet the Agency's needs. Health and environmental effects needs will be met through committed NHEERL scientists engaging with OPPTS leaders and staff to understand and to solve their problems. This combination of responsiveness and scientific challenge offers us the chance to make major contributions.

Process for Developing this Implementation Plan

In the Summer of 2003, an NHEERL Safe Pesticides/Safe Products steering committee (Table 1) was created with the purpose of developing a focused NHEERL SP^2 research program. The Steering Committee aims were to:

(i) Develop a "strawman" proposal for the workgroups by identifying the critical paths and milestones to the targets identified in the MYP
(ii) Develop research project descriptions to achieve the milestones
(iii) Periodically review the research findings
(iv) Adjust targets/milestones/projects as appropriate.

The first task of the Steering Committee was to define the essential science questions that must be resolved to accomplish the four long-term goals (see box page 1) and to identify draft LTG-specific critical paths of research to address these scientific challenges. These critical paths provide the framework for the NHEERL research implementation program to accomplish the LTGs.

The second step was to identify and recruit appropriate staff to serve on LTG-specific workgroups to refine the critical paths and define the research program for each LTG. The workgroups consisted of steering committee members (one or more served as workgroup leads) and additional NHEERL, OPPTS, staff (Table 2). By broadening participation on the workgroups we hoped to enhance buy-in from scientific staff and to make certain we established the most effective team possible for developing and implementing the NHEERL Research Program for OPPTS.

Table 1. NHEERL SP^2 Implementation Plan Steering Committee Members	
Organization	**Representative**
NHEERL	
ECD	Larry Claxton / Ann Richard
ETD	MaryJane Selgrade/ Woody Setzer
HSD	Rebecca Calderon /
NTD	**Kevin Crofton** / Ginger Moser
RTD	Sally Darney / Chris Lau
AED	**Tim Gleason** / Diane Nacci
GED	Mike Hemmer / Cal Walker
MED	Patricia Schmieder / Rick Bennett
WED	Anne Fairbrother/ Tom Pfleeger
ALD	Jack Fowle
OPPTS	
OPPT	Jennifer Seed
OPP	Steve Bradbury/Randy Perfetti
OSCP	Mary Belefski
Other ORD Labs & Centers	
NERL	Larry Burns (Athens), Ross Highsmith (RTP)
NRMRL	Greg Sayles
Note: Steering committee leads are in bold type	

The third step was to draft the plan. This was accomplished through two workshops and a variety of separate LTG specific workgroup activities. The first workshop was held January 21-23, 2004 in Research Triangle Park, NC. It's purpose was to engage in a dialog between the NHEERL and OPPTS partners to identify the problems facing OPPTS, the scientific questions they raise and to begin to focus on the highest priority areas to produce an implementation plan that will produce a research program that is on a critical path to meet OPPTS major needs. The workshop provided top down guidance from senior managers from both NHEERL and OPPTS and bottom up creativity from the workshop participants who came not only from NHEERL and OPPTS but from ORD's National Exposure Research Laboratory and from EPA's Region 10 as well. The first workshop also provided an opportunity for the NHEERL scientists to better know their partners in OPPTS, not only personally, but in terms of their business, their tools and their problems. And from this knowledge it provided an opportunity to begin to define the problems facing OPPTS in terms of the technical issues and scientific questions they face and to translate these into research that might be done to help. The focus on how OPPTS does business, as well as on the problems they face, provided a basis to define not only what research would be done and how, but also to begin to develop a critical path for the research. It also helped define criteria to evaluate whether the proposed research is merely relevant in some way to OPPTS' needs or whether it is truly responsive to their needs and thus likely to help solve their problems directly. The clear message was that if the work is relevant but not responsive it does not belong in the Implementation Plan. The agenda and workshop

The workshops provided top down guidance from senior managers from both NHEERL and OPPTS and bottom up creativity from the workshop participants

Table 2. SP2 - Long-Term Goal Workgroup Members

LTG	Co-Leads & Member		
1	Bill Mundy (Co-Lead, NTD) Pat Schmieder (Co-Lead, MED) Bill Mundy (Co-Lead, NTD) Pat Schmieder (Co-Lead, MED) Sally Darney (RTD) Jeff Welch (RTD) Ralph Cooper (RTD) Tim Shafer (NTD) Ginger Moser (NTD) Richard Wiggins (NTD) Ann Richard (ECD) Dr. Sheau-Fung Thai (ECD)	David M. DeMarini (ECD) Michael Hemmer (GED) Calvin Walker (GED) Lesley Mills (AED) Rod Johnson (MED) Ralph Smialowicz (ETD) Dave Thomas (ETD) MaryJane Selgrade (ETD) Kathy Anitole (OPPT/RAD) Ron Ward (OPPT/RAD) Yintak Woo (OPPT/RAD) Maggie Wilson(OPPT/RAD)	Cathy Fehrenbacher (OPPT/EETD) Kathleen Raffaele (OPP/HED) Kathryn Boyle (OPP/RD) Princess Campbell (OPP/RD) Jonathan Chen (OPP/AD) Jean Holmes (OPP/EFED) Jackie McQueen (ORD/OSP) Greg Susanke (ORD/OSP) Karen Hamernik (OSCP) EFED Backups: M. Rexrode N. Mastoda
2	Diane Nacci (Co-Lead; AED), Anne Fairbrother (Co-Lead; WED) Jason Grear (AED) Steven Walters (AED) Suzanne Ayvazian (AED) Nathan Schumaker (WED)	Rick Bennett (MED) John Nichols (MED) Chris Russom (MED) Foster Mayer (GED) Mace Barron (GED) Don Rodier (OPPT/RAD)	Ed Odenkirchen (OPP/EFED) *Ingrid Sunzenauer (OPP/EFED)* Craig Barber (NERL/ERD) Ann Pitchford (NERL/ESD) Lawrence Burns (NERL/ERD) Les Touart, (OSCP)
3	Ann Fairbrother (Co-Lead, WED) MaryJane Selgrade (Co-Lead, ETD) Phil Sayre (OPPT)	Jeff Evans (OPP/HED) Melissa Kramer (OSCP) *Winnie Roberts (OSCP)* Sandra Bird (NERL/ERD)	Susan Richardson (NERL/ERD) Mark Bagley (NERL/EERD) Greg Toth (NERL/EERD) Tim Collette (NERL/ERD)
4	Chris Lau (Co-Lead; RTD) David Olszyk (Co-Lead, WED) Mary Gilbert (NTD) Robert MacPhail (NTD) Mitch Rosen (RTD) John Rogers (RTD)	Thomas Pfleeger (WED) Henry E. Lee (WED) Doug Kuehl (MED) Bob Luebke (ETD) Mike Hughes (ETD) Woody Setzer (ETD)	Rebecca Daiss (OPP/HED) Jennifer Seed (OPPT) *Mike Davy (OPP/EFED)** *Karl Arne (Region 10)* *Glenn Thursby (AED)* ** EFED back-up.*
Meeting Coordination Team: Ginger Moser (Lead; NTD), Ingrid Sunzenauer (OPP), Tim Gleason (AED), MaryJane Selgrade (ETD)			

OPPTS/OSCP = Office of Science Coordination and Policy
OPPTS/OPPT = Office of Pollution Prevention and Toxics
- OPPT/RAD = Risk Assessment Division
- OPPT/EETD = Economics, Exposure, & Technology Division

OPPTS/OPP = Office of Pesticide Programs
- OPP/HED = Health Effects Division
- OPP/AD = Antimicrobial Division
- OPP/RD = Registration Division
- OPP/EFED = Environmental Fate and Effects Division

NERL = National Exposure Research Laboratory
- NERL/EERD - Ecological Exposure Research Division
- NERL/ERD = Ecosystems Research Division

NHEERL Divisions
- AED = Atlantic Ecology Division
- ECD = Environmental Carcinogenesis Division
- ETD = Environmental Toxicology Division
- GED = Gulf Ecology Division
- MED = Mid-Continent Ecology Division
- NTD = Neurotoxicology Division
- RTD = Reproductive Toxicology Division
- WED = Western Ecology Division

participants for the January 2004 workshop are listed in Appendix D (see NHEERL SP2 website - http://www.nheerl.epa.gov/nheerl_science/pesticide_implementation/).

A second workshop was held January 5, 2005 in Research Triangle Park, NC to sharpen the focus of the work being proposed under LTG 1 of the Implementa-tion Plan (Appendix E - see NHEERL SP2 website at: http://www.nheerl.epa.gov/nheerl_science/ pesticide_implementation/). The theme was "A Day In the Life of OPP" as the discussions were designed to provide NHEERL researchers with a better understanding of OPPTS' needs for research to help prioritize and rank chemicals for testing and to enhance the interpretation of data with particular emphasis on the Pesticide Programs. OPP staff provided an overview of:

a) OPP's mission and structure
b) Pesticide registration and re-registration
c) Risk management decision framework
d) Data requirements
e) Basis components of a risk assessment (i.e. problem formulation, analysis and risk characterization)

This was followed by presentations on the operations of the major OPP divisions, their specific needs and a comparison of the different types of risk assessments conducted by OPP. These discussions helped NHEERL researchers better understand the problems facing OPP in terms of the technical issues and scientific questions they face, and it facilitated partnerships between NHEERL and OPP staff to work together to translate the science questions stemming from OPP's problems into specific research approaches to help solve these problems.

The final step in the development of this plan was for the steering committee to integrate the output of the four workgroups into this draft final NHEERL Multi-Year Implementation Plan (MYIP) which is being sent to NHEERL and OPPTS Senior Management for review. OPPTS senior management is being asked about the extent to which we have been responsive to their needs in terms of how well we have correctly identified their problems and designed a research program that, if successful, would help resolve these problems by providing OPPTS with tools and information they can use in their operations.

We are asking senior NHEERL managers about the extent to which we have proposed a program that identifies the "right science" to meet OPPTS' needs and a plan to do the "science right". Additionally, they are being asked about the extent to which the proposed efforts complement the work done under the other NHEERL Multiyear Plans and about the feasibility of conducting the research given NHEERL's anticipated resources.

The steering committee will revise this plan based on these comments and will then submit it for external peer review. The plan will be finalized based once the external peer review comments have been received, hopefully by the end of calendar year 2005.

Coordination Across ORD

Key generic aspects of the questions addressed by this plan are not unique to the issues addressed in SP^2. The Computational Toxicology program and other programs, such as the Candidate Contaminant List research under the screening and testing efforts underway in the Endocrine Disrupting Chemical (EDC), and Human Health MYIP, as well as the research supported by the computational toxicology framework and the Drinking Water MYP share similar goals with respect to their focus on prioritizing and ranking chemicals for testing, and making the testing

process more efficient.

OPPTS plays a leading role in regulatory risk assessment in EPA and many of the research needs require very large research efforts which are intrinsic to the Agency business of environmental protection and often solve similar problems faced by other EPA offices. To provide that strategic direction for the work conducted under the Safe Pesticides Safe Products Multiyear Plan (MYP) the ORD Science Council held a series of meetings with the senior leadership of OPPTS to identify the major scientific impediments in the OPPTS regulatory programs.

OPPTS plays a leading role in regulatory risk assessment in EPA. Many of their research needs require very large research efforts which are intrinsic to the Agency business of environmental protection and often solve similar problems faced by other EPA offices.

To avoid duplication with efforts underway in other MYPs, a crosswalk between the strategic OPPTS needs and all of ORD multi-year plans led to the identification of major areas which were not addressed by other MYPs and where Safe Pesticides Safe Products resources for research should be focused. Those research areas are nested in the appropriate long-term research goals of the Safe Pesticides Safe Products Multi-Year Plan as illustrated in Figure 1. SP2 resources are intended to complement even larger core research efforts found in Goal 4 which also addresses many OPPTS needs. The four long-term goals comprising the SP2 MYP and this NHEERL SP2 Implementation Plan are shown as major OPPTS problem areas not being met by other MYPs in Figure 1. The remainder of this Implementation Plan describes how NHEERL has aligned its SP2 research efforts to respond to these unmet needs.

In developing this plan we have focused on developing the right science to answer questions being posed by OPPTS' needs. We hope to benefit from the work conducted under the EDC, Human Health and Computational Toxicology programs by applying the results to the work conducted to meet OPPTS' needs and *vice versa*. Leveraging efforts across a number of MYPs allows more rapid progress in the areas of predictive toxicology. We have identified in a number of places in this document where programs are being 'co-funded' by the National Center for Computational Toxicology (see also: www.epa.gov/comptox/research_activities.html#new_start). We believe that the Key is to "Do the right science" and to use all the tools in the toolbox to "do the science right". The various ORD MYPs identify related work conducted under other MYPs by cross-walking the APMs and listing the relevant ones in italics.

One common comment we have received from a number of reviewers is that there are many aspects of this plan that are not well "integrated". Reviewers clearly identified a need to improve cross-divisional and cross-laboratory (e.g., NERL, CompTox) collaboration. This is a long standing issue in NHEERL that cannot be addressed in short order. We propose working with this version of the SP2 Implementation Plan, and at the same time identify areas for integration, develop approaches, and set in motion the processes that will improve integration as we move forward over time. For example, one area of potential integration is connecting the various developmental toxicology efforts (immunotox, neurotox, etc). We have proposed a focused workshop to develop a conceptual model for integrating these efforts into true coordinated, multi-disciplinary research programs that will solve critical Agency problems.

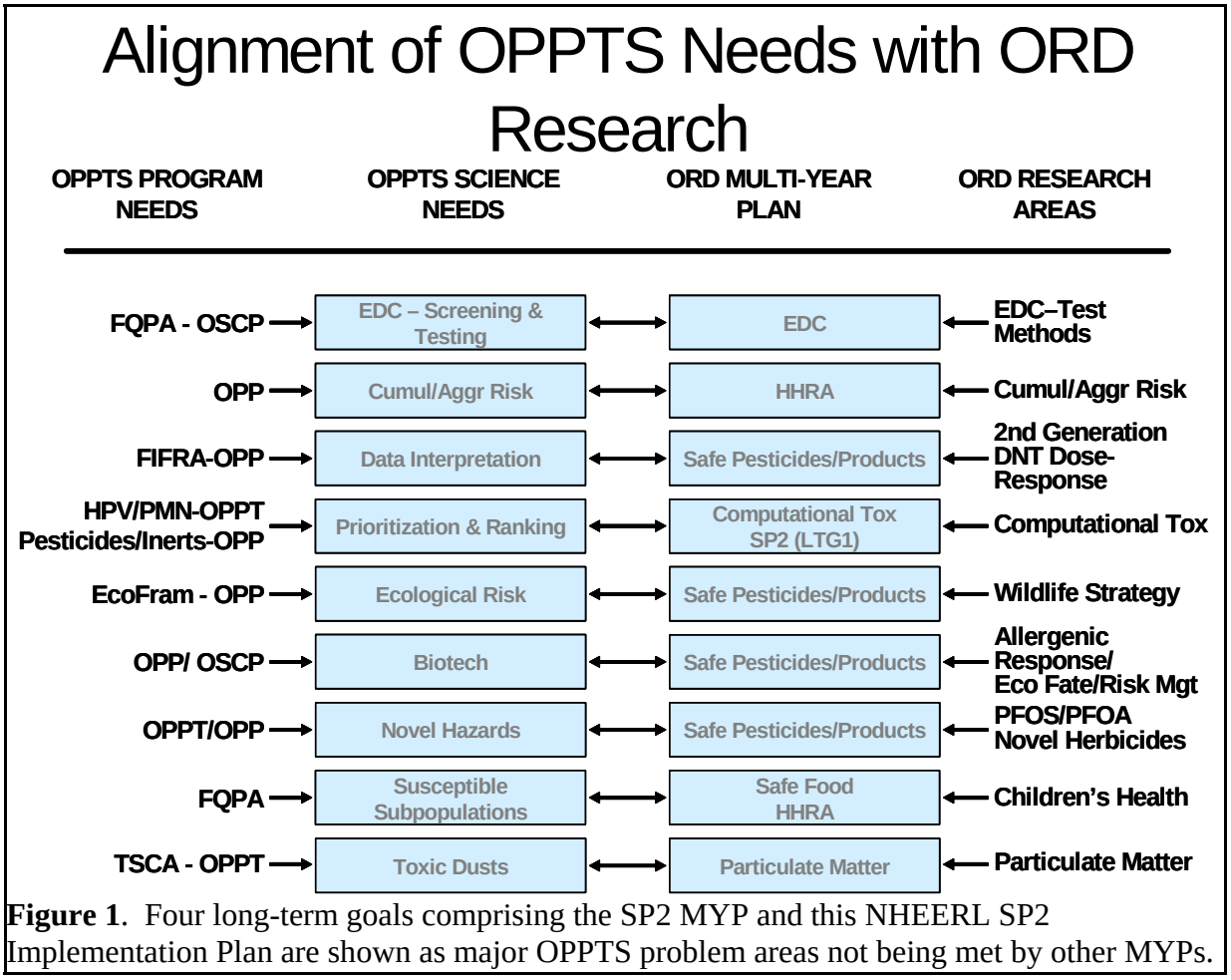

Figure 1. Four long-term goals comprising the SP2 MYP and this NHEERL SP2 Implementation Plan are shown as major OPPTS problem areas not being met by other MYPs.

Long Term Goal 1. To provide EPA with predictive tools for prioritization of testing requirements and enhanced interpretation of exposure, hazard identification and dose-response information.

Issue

Substantial advances in human health and ecological risk assessment have been achieved by ORD and OPPTS partnerships; however, significant challenges remain. The lack of available data on the hazardous properties of chemicals, and the desire to gain efficiency while enhancing the quality of risk assessment and management of chemicals are driving forces behind implementation of TSCA, FIFRA, and FQPA. There is a need for information to address the risk assessment uncertainties derived from a lack of knowledge across chemical classes and/or adverse effects and outcomes of concern. The magnitude of the knowledge gaps and the desire of regulatory agencies and affected stakeholders to close the gaps as quickly as possible, however, preclude the employment of a traditional toxicity testing approach. Thus, the long-term solution to meeting this challenge will not be the generation of more data faster, but rather determining what specific effects data, for which chemicals, and which exposures is essential to assess and manage risks appropriately. In this context, the Agency requires sufficient, targeted, credible information from which to make decisions. Consistent with this view is the consideration of time and cost efficiencies associated with the generation and interpretation of toxicity data and the sound and responsible use of animals.

Of the issues facing OPPTS, the need to develop more efficient ways to screen and prioritize chemicals for testing to acquire sufficient, targeted, credible information for decision making is most pressing.

For chemicals that lack adequate data on toxicological and exposure potential (e.g., certain PMN and HPV chemicals; inert pesticide ingredients; anti-microbial pesticides) the challenge is to create the means to efficiently and credibly predict toxic potency and levels of exposure. These predictions will enable reasonable decisions to be made as to whether or not empirical studies are required to further refine a risk assessment to inform management decisions. Current approaches for screening and testing chemicals requires extensive resources. Therefore, priority setting approaches must be developed to determine the sequencing of chemicals or classes of chemicals to assess for a specified risk endpoint. Additionally, while extensive data sets are generated for many toxicity endpoints currently used in risk assessment, efficiency can be gained in using targeted testing to reduce critical uncertainty while minimizing resource utilization. The current inability to estimate endpoints sufficiently to set hypothesis-driven risk-based priorities is the result of a lack of understanding of pathways of toxicity and how they can be initiated by chemicals, as well as by a lack of methods to model the complex behavior of chemicals. Thus, of the issues facing OPPTS the need to develop more efficient ways to screen and prioritize chemicals for testing to acquire

sufficient, targeted, credible information for decision making is most pressing.

Long Term Goal- 1 (LTG1) of ORD/NHEERL's Safe Pesticides/Safe Products MYIP (SP^2) seeks to provide EPA with predictive tools for hypothesis-driven prioritization of testing requirements and enhanced interpretation of exposure, hazard identification, and dose-response information.

To overcome these gaps, and to move toward a more sustainable risk assessment paradigm to support TSCA, FIFRA, and FQPA decisions, Long Term Goal- 1 (LTG1) of ORD/NHEERL's Safe Pesticides/Safe Products MYIP (SP^2) seeks to provide EPA with predictive tools for hypothesis-driven prioritization of testing requirements and enhanced interpretation of exposure, hazard identification, and dose-response information. This will be accomplished through close collaboration between NHEERL scientists and OPPTS colleagues to ensure that planned research is designed to meet specific OPPTS challenges. In doing so, the research also complements the ORD Computational Toxicology (CompTox) Research Program which seeks to develop new computational tools and predictive models to better elucidate and diagnose toxicity pathways, as well as to link chemical sources to outcomes. The research in SP^2-LTG1 also builds upon the screening and testing efforts underway in the Endocrine Disrupting Chemical (EDC), and Human Health MYIP, as well as the computational toxicology framework (Figure 2) by applying tools, techniques and knowledge to problem-driven research in support of the major OPPTS needs.

The research proposed under LTG 1 addresses many needs outlined in the draft OPPTS white paper "A Sustainable Risk Assessment Paradigm to Support TSCA and FIFRA/FQPA Decisions: Closing the Scientific Gaps" (EPA internal document; Feb. 2005) contained in Appendix F (NHEERL SP2 website: http://www.nheerl.epa.gov/nheerl_science/pesticide_implementation/) . Specifically, the research under LTG1 seeks to assist OPPTS in meeting the challenge of developing the means to move, in a scientifically credible and transparent manner, from a paradigm that requires extensive hazard testing, followed by the elimination of information not relevant to the assessment, to a paradigm that provides the means to use a risk-based, hypothesis-driven approach to identify the specific in vivo information most relevant to an assessment. This implementation plan recognizes that this fundamental paradigm shift is essentially the same for 'data rich' and 'data poor' chemicals across OPPTS programs, so NHEERL research will focus on

The proposed research addresses many needs outlined in the draft OPPTS white paper "A Sustainable Risk Assessment Paradigm to Support TSCA and FIFRA/FQPA Decisions: Closing the Scientific Gaps"

(i) priority setting and screening,
(ii) facilitating database development, and
(iii) enhancing data interpretation in moving toward a more hypothesis-driven risk assessment paradigm.

Understanding toxicity pathways, from initiating events to adverse outcomes, underlies NHEERL's LTG1 research approaches as a foundation to develop targeted testing designs that estimate *in vivo* potency for endpoints of interest. Our goal for this research is that scientific advances will, over the longer term, allow increasingly quantitative, efficient, and diagnostic approaches for assessing risks.

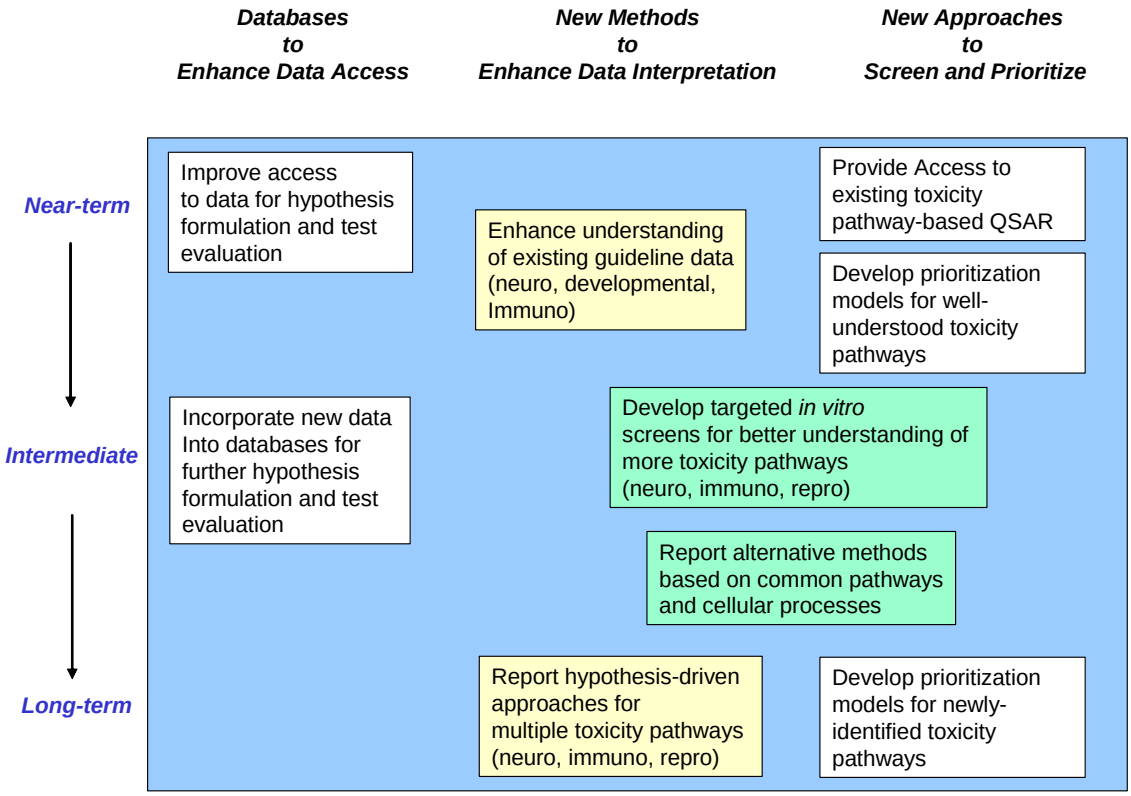

Figure 2. LTG1 Approach

Approach

Figure 3 presents the components and relative timing of NHEERL's efforts to more efficiently identify hazards using an hypothesis-driven approach to assess risk, and to better identify which chemicals should be prioritized for testing and to identify those which have adequate data base or for which no identifiable risk is likely. LTG 1 research is designed to build on an understanding of toxicity pathways to provide the critical links between chemical exposure and initiating events, biochemical and physiological and toxicological alterations, and, ultimately, adverse effects. The studies that will be conducted under LTG 1 are summarized in Table 3 and the project descriptions that follow. More detailed project descriptions are found in Appendix A on the (NHEERL website at: http://www.nheerl.epa.gov/nheerl_science/pesticide_implementation). Each project description includes an assessment of the impact additional resources would have on the project.

Databases - LTG1 research is planned to sequentially address the needs of OPPTS, consistent with the OPPTS white-paper timeline. As described previously, database development projects are proposed to make the most efficient use of existing OPP registrant data submitted under a wide variety of testing guidelines. The large quantity of guideline study data has the potential to advance the understanding of toxicity pathways by providing *in vivo* outcome information

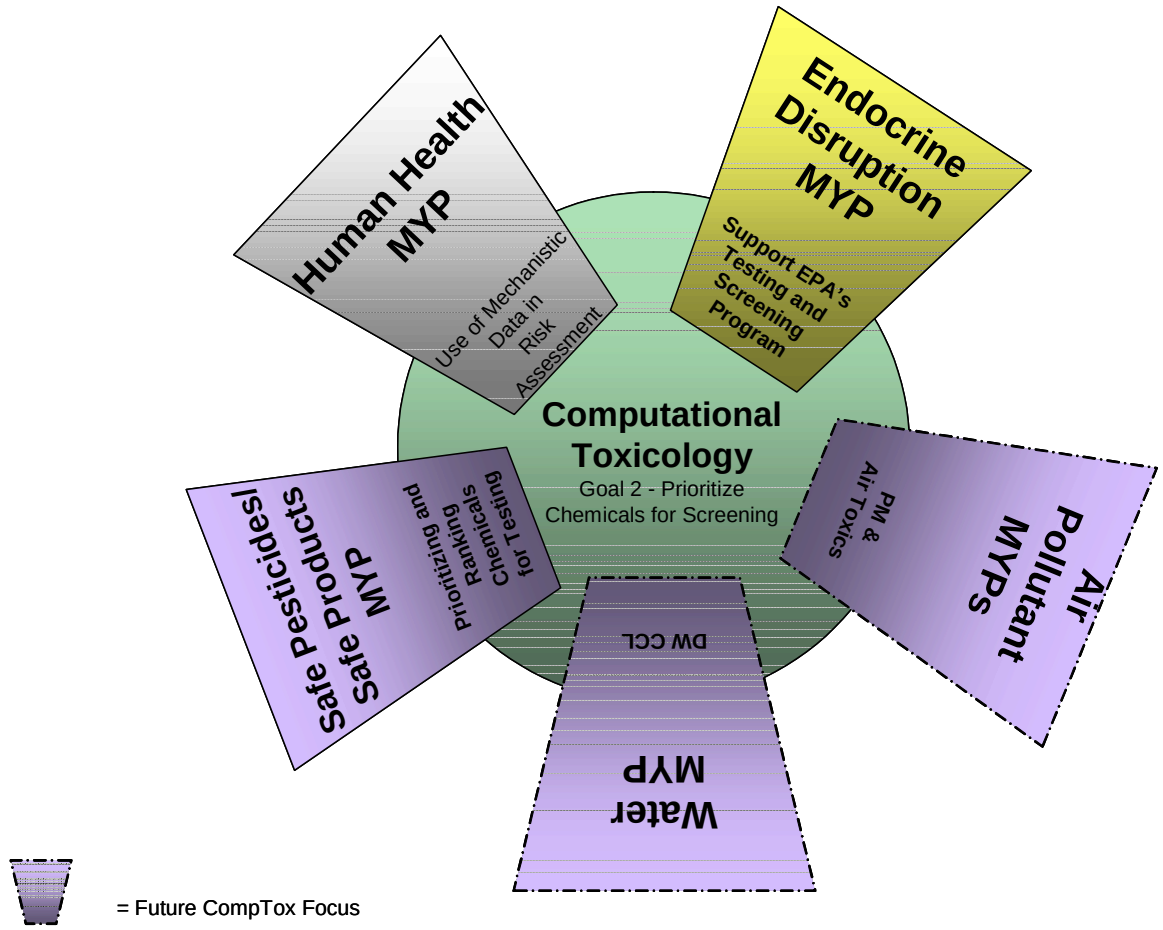

Figure 3. Relationship of prioritization and screening efforts across ORD programs.

collected under standardized guidelines. However, to reach this potential the data need to be widely accessible and searchable. LTG1 research proposals address these needs for toxicity endpoints, and chemical metabolism and degradation pathways. Outputs of the database efforts will contribute both to enhancing data interpretation and to prioritization and screening. The first step of the approach is to identify where data already exists and to make it more easily accessible for evaluation by both risk assessors and researchers. Thus, building and populating well-designed databases is a near-term emphasis because it is the key to making progress in the intermediate and longer-term. Searchable databases contribute in multiple ways to LTG 1 projects by:

a) Greatly assisting data interpretation by allowing efficient access to information otherwise unevaluated due to inaccessibility or simply not knowing it exists
b) Allowing records to be grouped by user-defined descriptors to facilitate evaluation of data in new ways and to discover associations previously not examined
c) Providing better access to program office data to allow identification of critical linkages in toxicity pathways thus enhancing interpretability of *in vitro* and biomarker data linked to adverse outcomes
d) Assessing where knowledge gaps exist, and
e) Allow examination of where correlations can and cannot be made across endpoint

measures, across chemicals, and across species

It is envisioned that NHEERL will work with OPPTS risk assessors and others to design databases that will:

a) Allow better understanding of existing data
b) Allow systematic evaluations of some of the data resulting from newer guidelines and protocols that have as yet not been evaluated
c) Facilitate comparisons of information available in current tests with newly developed tests to guide the development of new testing approaches in a hypothesis-driven manner to target where efficiencies or enhanced interpretation can be gained.

Where a toxicity pathway is sufficiently understood, and where assays are available for that pathway, systematically harnessing the existing knowledge-base needed may provide all that is needed for the development of more realistic scientific basis to assess risk. In that vein, toxicity

Table 3. Summary of NHEERL LTG 1 Program Projects

Program Project Area 1: Enhancing Data Interpretation			
Approach	Title	Partners	Contact/Lead
A: Databases for Hypothesis Formulation and Testing	Project 1. Designing Toxic Effects Databases for Health and Ecological Effects Data: A Demonstration Project Using DNT Health Effects Endpoints.	NHEERL/NTD; OPP/HED; NHEERL/MED	S. Padilla, NTD
	Project 2. Designing a Pesticide Metabolic Pathway Database for Registrant Submitted Health Effects Data*	NHEERL/MED; NERL/ERD-Athens; EPA Cooperative Agreement; OPP/HED	P. Schmieder, MED
	Project 3. Designing a Pesticide Degradates Database for Registrant Submitted Ecological Effects Data*	NHEERL/MED; NERL/ERD-Athens; EPA Cooperative Agreement; OPP/EFED	P. Schmieder, MED
B. Enhanced Interpretation of Existing Guideline Data	Project 1. Evaluate Data from Current Developmental Neurotoxicology Testing (DNT) Guidelines - Data Reviews	NHEERL/NTD; OPP/HED	K. Crofton, NTD
C. Interpreting New Methods, New Data	Project 1. Development of Cell Culture and Biomarker Based Screening Methods for Non-Endocrine Reproductive Toxicity	NHEERL/RTD	J. Welch, RTD
	Project 2. Determination of Sensitive Immune Function Endpoints for Identification of Adult and Developmental Immunotoxicity	NHEERL/ETD	R. Smialowicz, ETD

Table 3. Summary of NHEERL LTG 1 Program Projects (con't)			
Program Project Area 2: Screening and Prioritization			
A. Models	Project 1. Developing Toxicity Pathway-Based QSARs for Prioritization Within Large Chemical Lists.	NHEERL/MED; OPP/RD; OPP/AD; OPP/EFED	P. Schmieder, MED
	Project 2. Providing Access to Peer Reviewed Literature and Mode of Action Based QSAR Models: Assessment Tools for Ecological Risk (ASTER)*	NHEERL/MED; OPP/EFED	C. Russom, MED
	Project 3. Simulating Metabolism to Enhance Effects Modeling	NHEERL/MED; NERL/ERD-Athens; EPA Cooperative Agreement	P. Schmieder, MED
B. Methods	Project A. Alternative Methods for Screening and Prioritization of Developmental Neurotoxicants	NHEERL/NTD	W. Mundy, NTD
	Project B. Toxicity Pathway-Specific Protein Expression Models for Chemical Screening and Prioritization*	NHEERL/GED	M. Hemmer, GED

*Co-funded, collaborative effort with National Center for Computational Toxicology

pathway-specific QSARs for prioritization and screening are under development for currently well-defined pathways building on the OPP historical database. In the mid- and longer- term research is underway to demonstrate the approach and build the tools needed which can be applied in the future as more key pathways are elucidated.

New Methods to Enhance Data Interpretation – Efficient access to existing data to identify similar acting chemicals based on toxicological outcome can be achieved by developing searchable databases. Collaborative efforts between NHEERL and OPP risk assessors under LTG1 will focus on outcomes that are of high priority to enhance interpretation of data and that optimally leverage existing NHEERL research approaches and expertise, so that both risk assessors and researchers maximally benefit from these efforts. LTG1 database projects also support the need to enhance our current ability to interpret many types of data submitted under existing guidelines. In some instances sufficient data have only recently become available as relatively new guidelines were introduced over the last several year. Areas are identified where extensive evaluations of newer datasets and examination of test protocols by ORD and OPPTS collaborators will be undertaken to help inform current risk evaluations and to direct the development of hypothesis-driven predictive, diagnostic markers (biochemical, proteomic, genomic) for given risk endpoints (e.g., immunotoxicity, neurotoxicity, reproductive effects). Hypothesis-driven predictive, diagnostic markers will help us learn if we use the results to form the basis for further targeted testing. The goal is an increased understanding of toxicity pathways to guide testing and to generate high-quality datasets.

Where toxicity pathways are not sufficiently understood (e.g, where there are critical knowledge gaps in the continuum from chemical initiation of the toxicological process to its

manifestation as a whole organism adverse outcome) research will be undertaken in concert with NHEERL's core research programs in Human Health, Endocrine Disruption and Computational Toxicology to assess current understanding of the events leading to adverse effects, to identify critical knowledge gaps, to understand commonalities in toxic pathways to develop integrative new methods that provide information on multiple effects, and to develop *in vitro* assay approaches for rapid screening.

New Approaches to Screen and Prioritize – High quality data are also essential to the development of predictive models, such as structure-activity relationships (QSARs), needed to prioritize chemicals for hypothesis-driven regulatory testing. Where large numbers of chemicals exist that need to be assessed based on little or no measured data, QSAR predictions can be used to prioritize which should be tested, when, and for which endpoints.

Such approaches are needed to form the basis for targeted regulatory testing to increase risk assessment efficiency, but is only possible where toxicity pathways are well-defined and where assays are available for sufficient strategic testing to build applicable models. Strategies to systematically test within large chemical inventories are included in LTG 1 to minimize the collection of redundant information and to maximize the understanding of the attributes of chemical structural that initiate specific biological interactions. Targeted testing approaches in LTG1 seek to incorporate new *in vivo protocols* that provide data with less uncertainty, and/or to provide *in vitro* assays that in high-throughput systems (HTPS) because they are linked to the *in vivo* endpoint.

Tools needed for screening and prioritization follow a logical progression from early method/assay development, through the application of an assay or suites of assays in a diagnostic manner to elucidate toxicity pathways, to the systematic testing of multiple chemicals to identify groups that initiate toxicity in a common way. Once testing can be done within a pathway, chemical testing can be focused to determine features of chemical structural that facilitate chemical-biological interactions. Quantifying the structural requirements through which a chemical initiates a toxicity pathway allows the prediction of probable toxic interactions for a given chemical. As the knowledge base grows, it can be used as a basis to prioritize testing requirements based on likelihood of causing effects.

Short and intermediate-term research in this area focuses on providing better access to existing models, developing models where toxicity pathways are well understood, and providing tools and approaches that can be applied in the longer term as understanding of additional pathways allow. Long-term research in this area will attempt to integrate data generated from intermediate-term projects including in vitro screens and genomic and proteomic approaches with new knowledge of toxicity pathways to develop predictive models that specifically address data gaps in the risk assessment process. Systematically collected assay data in short- and intermediate-term projects will, using tools and approaches currently under development, serve as the basis for toxicity pathway-based QSAR prioritization protocols in the future for the major endpoints of regulatory concern. The gradual development of a library of pathway-specific models will eventually allow the prioritization of testing endpoints.

Impact of LTG 1 Research

The combination of strategic approaches to chemical selection, efficient testing using HTPS to generate diagnostic information, and the incorporation of systematically collected test data into structure-based predictive models, will ultimately be combined into screening and prioritization approaches for OPPTS that will minimize resources necessary to provide sufficient, targeted, credible information for efficient risk assessment.

Timing

Short-, Intermediate-, and Long-term projects are included in each of the focus areas and are as consistent as possible with the timeline proposed in the OPPTS white paper. In several instances, intermediate and longer-term progress is dependent upon initial accomplishments. In some instances resource availability is a deciding factor.

Relationship Between SP2 LTG1 and the Computational Toxicology Research Program

As noted in the introduction, there are close relationships between the work proposed in this plan and work underway in other ORD research programs. No where is this more true than for LTG 1 which relies heavily on being able to apply advances in computational toxicology (CT) to accomplish its objectives. The CT program will also benefit from work conducted under LTG 1 because early success stories that meet near- to mid-term programmatic needs in specific areas (e.g., prioritization/screening research in SP^2, LTG1) will demonstrate the broader applicability of problem-driven CT approaches within the Agency. Applications in the area of priority setting and screening with the CT framework are readily aligned with TSCA and FIFRA needs to provide opportunities to move forward by demonstrating the practical feasability of instituting a broader Agency effort to incorporate CT approaches across the research programs to improve the science informing Agency decisions.

NHEERL/OPPTS Partnership

Traditionally researchers at EPA have worked with their program office counterparts in a client relationship. However, a partnership between NHEERL and OPPTS is critical if we are to plan and implement a research effort that ensures critical programmatic needs will be met in an appropriate sequence and time frame. Figure 4 explicitly diagrams the partnerships that must be established to link NHEERL research activities with corresponding OPPTS activities to ensure that regulatory advances are being made in concert with the anticipated and actual development of new scientific tools. This can only be accomplished by initial problem formulation and research planning followed by ongoing dialog and interactions as the research and the regulatory development proceed. Throughout the lifecycle of a program, ORD, NHEERL and OPPTS senior management involvement and review of the strategic and tactical direction and progress is needed to ensure the array of staff and resources across various implementation efforts achieve a holistic program that includes appropriate support and expertise to address technical support needs that arise throughout the regulatory process.

LTG 1 Program Project Areas

Five program project areas have been identified under LTG 1 in this plan. They are designed to enhance data interpretation and provide increased capacity for screening and prioritizing inventories of previously untested chemicals. The projects are organized as follows and discussed below.

1. Enhancing Data Interpretation (Program Project Area 1)
    a. Databases for hypothesis formulation and testing
    b. Enhanced Interpretation of Existing Guideline Data
    c. Interpreting New Methods, New Data
2. Screening and Prioritization (Program Project Area 2)
    a. Models
    b. Methods

More detailed descriptions of these projects are found in Appendix A on the NHEERL website:
http://www.nheerl.epa.gov/nheerl_science/pesticide_implementation/

Critical Path for Implementing the Computational Toxicology Framework in Regulatory Decision Making: Risk-Based Priority Setting for EDCs, Pesticide Inert Ingredients, HPVs and Antimicrobial Pesticides

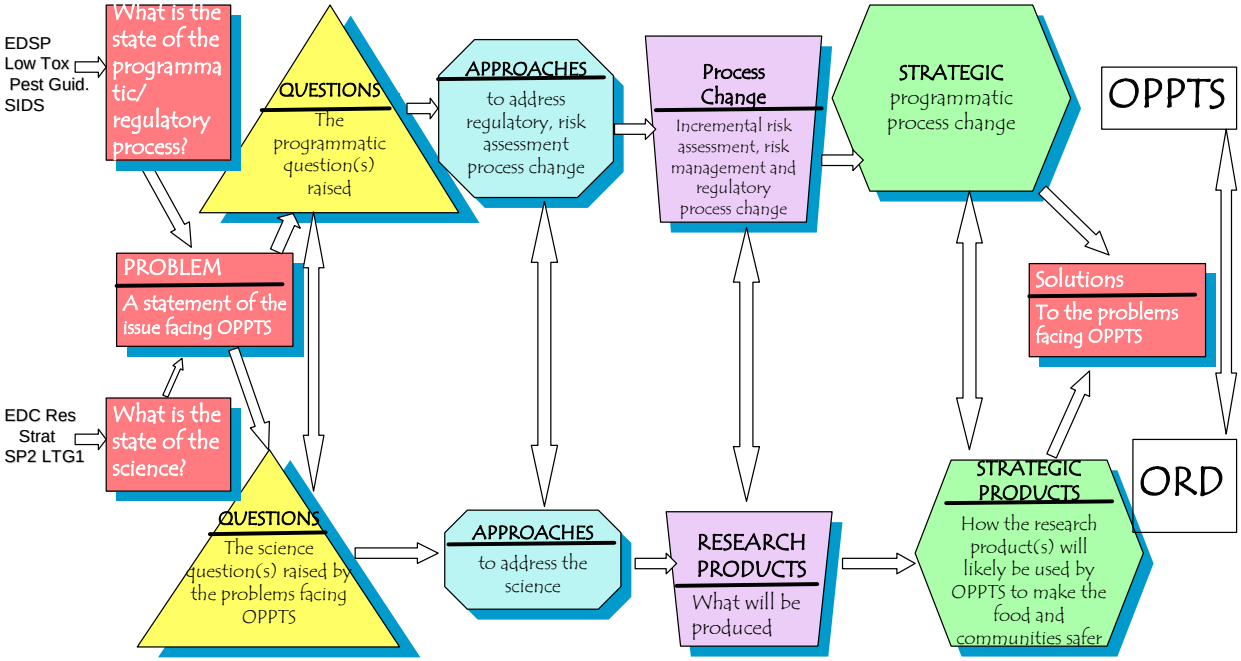

Figure 4

Program Project Area 1: Enhancing Data Interpretation

Approach A: Databases for Hypothesis Formulation and Testing

Project 1: Designing Toxic Effects Databases for Health and Ecological Effects Data: A Demonstration Project Using DNT Health Effects Endpoints

1. What are the key OPPTS problems this research effort will address?

Access to existing health effects data is slow and usually not complete. The data are not in easily accessible formats, but in non-searchable text files or exist as paper copies. To inform the human health risk assessment process, OPPTS requires rapid access to comprehensive information on the toxic effects of chemicals. In addition, as ORD researchers develop alternative test methods, rapid and comprehensive access to toxic effects data, including the open literature and OPP registrant submitted data, is needed.

2. What is the proposed research approach?

This pilot project will build the templates to incorporate developmental neurotoxicity data from the literature and OPP datasets into an inclusive, searchable database. It will be designed to meet the needs of OPPTS's risk assessors by providing ready access to chemical and toxicological information underlying past and current risk decisions, and augment the development of alternative test methods by allowing ORD researchers to link mechanistic indicators of toxicity to adverse outcomes of concern. This work will be a collaborative effort between OPP and ORD to identify the endpoint data and associated study information to be included in a searchable database. This database will be built upon the ECOTOX and ISTEP database structures. The templates will include fields suitable for information from both guideline and literature studies, as well as data from alternative test methods being developed in ORD.

3. How does this research support the conceptual model for addressing this LTG?

A database is information, information in a searchable format. As information accumulates patterns emerge, patterns that would not be apparent unless all the data were gathered together and viewed as a whole. These patterns will allow OPP or ORD scientists to predict the toxicity of other, unknown chemicals, allowing for "prioritization of testing requirements." All these issues directly address this Long Term Goal.

4. If this project is successful, what products or tools will result from the effort?

This effort will provide a template for a database to incorporate health effects data from developmental neurotoxicity studies, and could serve as a model for similar databases for other endpoints (e.g. reprotox, immunotox). With appropriate support, the second phase of this project will populate the database with information from an initial set of chemicals used as positive controls for validation of alternative methods for DNT (Project Area B; Approach 2). This project can also be expanded to include data from other Program Projects under LTG1 such as immunotoxicity (A3, project C).

APM. Report on list of chemicals and associated data describing positive and negative controls for developmental neurotoxicity	FY06
APM. Pilot for searchable database which encodes information from guideline studies, literature, and alternative methods for developmental neurotoxicity	FY07

Project 2: Design a Pesticide Metabolic Pathway Database for Registrant Submitted Health Effects Data

1. **What are the key OPPTS problems this research effort will address?**

To inform the risk assessment process, OPPTS requires information on the toxic effects of pesticide metabolites and the unmetabolized parent chemical. Currently OPP receives metabolic maps with registrant metabolism study data submissions. There is no current efficient manner to access previously submitted maps for similar chemicals to help with assessment of new chemicals. Information from past studies can assess the likelihood that all potentially toxic metabolites have been considered, More efficient use of existing data is needed to meet the challenge of resource limitations in meeting aggressive assessment deadlines. Additionally, without access to this data it is difficult to identify similarities in chemical metabolism and to formulate and test hypothesis for the types of chemicals that are of highest concern to EPA.

2) **What is the proposed research approach?**

ORD researchers ((NHEERL/MED; NERL/ERD-Athens) in collaboration with OPP/HED risk assessors, will design a database to provide access to currently unaccessible metabolic map information that exists in HED files. Existing software for the rapid and efficient depiction of metabolic maps (see associated Project 2.A.3) is being modified and enhanced to includes the ability to: depict hierarchical connection sequences of parent chemical and all listed metabolites; track radiolabel within a pathway and combine (or separate out) maps from associated radiolabel studies; identify all maps that contain a specific metabolite of concern; search for specific sub-structures of toxicological concern; and, compare complete maps across chemicals to find commonalities. Associated chemical identifiers and tracking information is included, as well as bioassay and analytical chemistry. Care will to taken to maximize efficiency in data import/encoding to the system; to design user friendly data searching, data exporting, etc. Early outputs of the project will demonstrate capabilities of the system by coding up metabolic maps for a sample set of pesticides selected by HED. This will allow identification of features needed to provide a system that is efficient, relatively easy to use, and that allows efficient recall and analysis of data by OPP risk assessors that was not previously possible.

3. How does this research support the conceptual model for addressing this LTG?

Maximal accessibility and use of existing data is supported by this effort. Access to metabolism data in a searchable format is key to understanding the role of metabolic activation in toxicity pathways, and to being able to generating targeted hypothesis that will address the highest priority uncertainties for the types of chemicals of most concern to the program offices.

4. If this project is successful, what products or tools will result from the effort?

Metabolism database prototype software, coding templates, and a demonstration database will be provided, designed to allow efficient assessment of the potential for metabolic activation to play a significant role in a chemical risk assessment.

APM. Deliver design templates for comprehensive and efficient capture of HED metabolism pathways, prototype software, and demonstration database to evaluate data capture and output.	FY06
APM. APM. Deliver design templates for comprehensive and efficient capture of HED metabolism pathways, prototype software, and demonstration database to evaluate data capture and output.	FY07

Project 3: Designing a Pesticide Degradates Database for Registrant Submitted Ecological Effects Data

1. **What are the key OPPTS problems this research effort will address?**

OPP/EFED must assess the impact of chemical degradates in the environment and use that information in determining human health and ecological risk. Access to degradate pathways is a key step in understanding the role of degradation processes in environmental toxicities. The information is used in interpreting bioassay data and in developing systems to predict these effects. The inability to readily access and compare degradate pathway information with metabolism pathway and residue information, across similar chemicals within OPP, limits the ability to identify chemical analogues with similar structures and activities.

2) What is the proposed research approach?

ORD researchers in collaboration with OPP/EFED staff are designing a chemical degradate pathway database to provide ready access to pathways of degradation associated with identified reaction types, for specified bioassay conditions across all study types and chemicals of concern. The system can be used to identify degradates of concern, to find other documented occurrences of the same degradate or the sub-structure of interest, and to evaluate the association between degradate and parent chemical structures, leading to better understanding of reaction types. The database is designed in collaboration with OPP to ensure that data pertinent to risk assessment questions are captured and encoded to allow efficient search and retrieval, and to build upon but not duplicate information in current systems. Care is taken to maximize efficiencies in data import and to design user friendly data searching and export features. Initial outputs of the project will demonstrate capabilities of the system by coding up degradate maps for a sample set of study types selected by OPP. This will allow researchers and risk assessors to identify features needed to provide a system that is efficient, relatively easy to use, and that allows acquisition and analysis of pathways by OPP risk assessors that was not previously achievable. Progress on this effort is a prerequisite to determining the approach and resource commitment needed to develop a system capable of predicting degradation pathways of OPP/EFED concern. (see Projects 1. A. 2. and 2.A.3).

3. How does this research support the conceptual model for addressing this LTG?

Electronically-stored structures of degradates which include pathway connectivity is a necessary first step to developing and applying genetic algorithms and related artificial intelligence systems to predict environmental degradation pathways. The development of searchable databases is key to efficient use of existing information and moving toward a new paradigm based on hypothesis-driven testing and prioritization.

4. If this project is successful, what products or tools will result from the effort?

A database that will allow generating and testing hypothesis of common reaction types across chemical structures. This information provides the basis for selection of analogues and provides the data needed as a foundation for developing procedures to simulate degradation processes.

APM. Report on the extent of degradate map availability across chemicals and degradate study types.	FY06
APM. Deliver design templates for comprehensive and efficient data capture of EFED degradate maps, prototype software, and demonstration database to evaluate data capture and output. (dependent upon results of above APM) .	FY07

Approach B: Enhanced Interpretation of Existing Guideline Data

Project 1: Evaluate Data from Current Developmental Neurotoxicology Testing (DNT) Guidelines - Data Reviews

1. **What are the key OPPTS problems this research effort will address?**

OPPTS currently has difficulties using and interpreting data from the Developmental Neurotoxicity Testing (DNT) guideline. The DNT has been criticized, both from within and outside the Agency, in terms of its overall sensitivity and usefulness as a screening method. Many criticisms currently being voiced against the current DNT are based on a subjective bias of the observers. Seldom are the criticisms bolstered with supportive data. These criticisms support the need to provide objective data to assess the strengths and limitations of the current testing battery.

2. What is the proposed research approach?

This project will evaluate and provide guidance on major issues regarding the methods used in the current Developmental Neurotoxicity Testing Guidelines. Study reports will be reviewed and summarized in a manner that will allow evaluation of the power and limitations of the data collected under current guidelines. Parameters such as variability, reliability, and sensitivity will be evaluated across studies and laboratories conducting the tests. There are collaborative efforts currently underway between the Neurotoxicology Division, NCEA, and the Office of Pesticide Programs and Toxic Substances to address these issues.

3. How does this research support the conceptual model for addressing this LTG?

This project will assist the Agency by enhancing interpretation of data from DNT studies. This is a basic tenant of LTG1. The outputs from the project will assist the Agency to revise the DNT guideline to provide better and more accurate data and increase the ability to interpret data. Evaluation of current data will also identify areas where hazard identification or data interpretation is problematic, and therefore assist in prioritization of research to develop new predictive, diagnostic markers developmental neurotoxicity.

4. **If this project is successful, what products or tools will result from the effort?**

Output from this project will include guidance documents for data requirements, standardized protocols and data evaluation, for use both within and outside the Agency. The outcome of this effort should be of immediate use to OPPTS in terms of data interpretation, requirements for future testing, and guidance for test method development and refinement.

APM. Report on the use of learning and memory testing in data from developmental neurotoxicity guideline.	FY07
APM. Report on the potential for maternal body weight changes to confound interpretation of the results from DNT studies	FY07
APM. Report on an evaluation of the use and relevance of the individual components of the DNT with recommendations for revisions	FY08

Project 2: Development of Cell Culture and Biomarker Based Screening Methods for Non-Endocrine Reproductive Toxicity

1. **What are the key OPPTS problems this research effort will address?**

The problem facing the program offices (OPPTS and OW), is the number of compounds that must be evaluated by the Agency for toxicity testing. While the current protocols are capable of identifying reproductive toxicants, they are time consuming, expensive, and require multi-generation animal studies. Development of high throughput cell culture based in vitro screening methods will allow the Agency a rapid and efficient means of prioritizing chemicals for further testing. Similarly, identifying biomarkers of reproductive toxicity will allow determination of reproductive risk to be made in one generation reducing both time, cost, and animal usage. Since in vitro methods to detect endocrine mediated reproductive toxicity are already being developed within the Agency, the creation of a cell culture and biomarker based screening system will complement ongoing test development by providing rapid and less expensive tests for non-endocrine mediated reproductive toxicity.

2. What is the proposed research approach?

This research will utilize Sertoli cell cultures challenged with a panel of known reproductive toxicants to identify insult induced effects in vitro. Sertoli cells serve as supporting nurse cells for the developing spermatozoa and are known to prematurely release germ cells in response to insults. Sertoli cell markers will be examined and their utility as endpoints for high throughput screening determined. Cell cultures will also be evaluated using genomic and proteomic methodology to identify novel biomarkers suitable for inclusion in the screening model. This approach will subsequently be extended to additional reproductive cell lines (i.e. epididymal cells), seminiferous tubules and intact animals to identify additional biomarkers. Emphasis will be on identifying markers with the potential to be used as in current one generation tier testing. Communication will be maintained with other screening and prioritization oriented projects in LTG1 to identify common research issues and avoid redundancy.

3. How does this research support the conceptual model for addressing this LTG?

Screening for reproductive effects using biomarkers and *in vitro* cell cultures will aid in prioritizing the testing of chemicals under review and characterization of new biomarkers will enhance interpretation of existing information on the effects of reproductive toxicants. This research will specifically approach the needs to 1) utilize emerging technology to develop rapid screening and prioritization tools; 2) identify new markers of effect that would increase our understanding of current information on reproductive toxicity.

4. If this project is successful, what products or tools will result from the effort?

A high throughput cell culture based tool for assessing testicular and epididymal insult would be provided to OPPTS for rapidly evaluating the potential reproductive toxicity of chemicals under review. Biomarker identification, while an integral part of methods development, will also provide additional endpoints that can be quickly integrated into the existing tier testing system and which will enhance the utility of current reproductive toxicity evaluations.

APM. Report on Sertoli and epididymal cell culture based screening assay	FY08
APM. Report on Biomarker identification, characterization and potential applicability to high throughput reproductive toxicity testing	FY10
APM. Report on assay fidelity in predicting reproductive toxicity	FY12

Project 3: Determination of Sensitive Immune Function Endpoints for Identification of Adult and Developmental Immunotoxicity

1. What are the key OPPTS problems this research effort will address?

OPPTS is faced with a significant challenge to identify and evaluate myriad man made chemicals which are found throughout the environment. Unfortunately, there is a plethora of environmental chemicals for which little or no data are available. While much effort has been invested in delineating the potential effects of many chemicals on reproductive, developmental, and neurological integrity, to name a few, a minimal effort has been exerted in the area of the immune system. As such, there is a need to assist OPPTS not only in the interpretation of the extant animal immunotoxicity data base, but also to identify the most robust and predictive functional assays currently available.

2. What is the proposed research approach?

The focus of this research will be to identify sensitive in vivo and in vitro approaches for recognizing and screening alterations of rodent immune function. The feasability of applying genomics as a screen for alterations of immune function (i.e., immuno-toxicogenomics) will be pursued. The potential increased susceptibility of the developing immune system, as well as the reproductive and central nervous systems, to environmental chemical exposure is a major concern given that these "sensitive" populations would be at greater risk. A significant need of OPPTS is an integrated testing scheme for developmental neurotoxicity, reproductive toxicity, and immunotoxicity, the latter of which is relatively more. Such an evaluation in a single study, is a viable, economical and productive means of determining developmental toxicity.

3. How does this research support the conceptual model for addressing this LTG?

The employment of *in vivo* and *in vitro* functional assays, along with the application of genomics, should provide a significant link to the identification of potentially undesirable environmental chemicals which impact the immune, reproductive and central nervous systems. A comparison of the sensitivity of *in vivo* and *in vitro* immune function results versus genomics should lead to the identification of the most sensitive metric(s) for immuno-, repro- and neuro-toxicity testing. Identification of increased potential susceptibility of the developing immune system will be an important goal. Accomplishment of these outcomes is a high priority. Presently, much more work and consequent data evaluation is required.

4. If this project is successful, what products or tools will result from the effort?

The identification of sensitive immune function endpoints (i.e., screens) which would serve as "red flags" for immunotoxicity associated with different environmental chemicals. The linkage of data generated in vivo and/or in vitro, along with the application of genomics, would serve as a high throughput metric. In addition, establishment of the consequences associated with chemical exposure during immune system development will be identified.

APM. Co-organize a workshop on the application of immunotoxicogenomics	FY05
APM. Initiate genomic approach to elucidate immunosuppression and hypersensitivity	FY10
APM. Initiate planning on "comptox" research for immunotoxic pathway	FY14

Program Project Area 2: Screening and Prioritization

Approach A: Models

Project 1: Develop Toxicity Pathway-Based QSAR for Prioritization Within Large Chemical Lists

1. **What are the key OPPTS problems this research effort will address?**

For OPPTS chemicals that lack adequate data on toxicological and exposure potential (e.g., certain PMN and HPV chemicals; inert pesticide ingredients; anti-microbial pesticides), the challenge is to create the means to efficiently and credibly predict toxic potency. The predictions will enable reasonable decisions to be made as to whether or not empirical studies are required to further refine a risk assessment, and will allow OPPTS to move toward a new assessment paradigm that targets specific effects data needs, for specific chemicals and exposures, that is essential to assess and manage risks appropriately. This research addresses the challenge to efficiently and credibly predict toxic potency for chemicals for which data is insufficient or completely lacking. It allows assessment of what adverse outcomes are most likely associated with a particular chemical structure by varying structure and measuring resultant activity within well-defined toxicity-pathways. The focus on initiating events within a toxicity pathway serves as the foundation to optimize *in vitro* assays and develop credible *in silico* approaches to estimate toxic potential, ultimately allowing ranking and prioritization of chemicals for their potential to elicit adverse outcomes and providing a means to prioritize testing requirements to increase efficiency in testing, test evaluations, and risk assessments.

2. What is the proposed research approach?

This pilot project will use a multi-faceted approach to determine the chemical structural requirements for initiation of distinct toxicity pathways. It incorporates QSAR-based hypothesis generation, strategic chemical selection for hypothesis testing, *in vitro* assay optimization and targeted testing, and QSAR evaluation and improvement for mechanistic classifications for OPP pesticidal inerts and antimicrobials, chemicals for which data is lacking and predictions welcomed. The approach is grounded in seeking mechanistic understanding of underlying chemical-biological interactions, and defining chemical similarity in terms of biological activity. Strategic selection of chemicals for testing is an essential component, given the numerous diverse chemicals for regulatory scrutiny. The immediate goal is to determine structural requirements for chemical binding to the estrogen receptor, a more complex interaction than previously appreciated. The larger objective is to present a process applicable to recurring issues surrounding determining structural attributes associated with toxicity and leading to adverse biological consequence. Determinations must be made with enough specificity to result in reliable predictions but broad applicability to numerous diverse chemicals. The process used strives for mechanistic interpretability and transparency, allowing evaluations of coverage within inventories to which models are applied.

3. How does this research support the conceptual model for addressing this LTG?

The research develops and applies *in vitro* and *in silico* techniques for prioritization and ranking within a regulatory context for a defined toxicity pathway. Model development and applicability is provided with guidance on use to enhance data interpretation.

4. If this project is successful, what products or tools will result from the effort?

Products include guidance on development of QSAR prioritization models for toxic effects endpoint in the context of well-defined toxicity pathways, and demonstrated application of the methods for OPP chemical lists for which testing priorities are requested.

APM. Report on the development of a systematic approach for the identification of strategic testing sequences for pesticides.	FY06
APM. Report on fish in vitro test results and ranking for an OPP inventory.	FY06
APM. Report on inventory ranking for additional OPP lists.	FY08
APM. Report on progress of prioritization approaches for inventories and endpoints.	FY10

Project 2: Providing Access to Peer Reviewed Literature and Mode of Action Based QSAR Models: Assessment Tools for Ecological Risk (ASTER)

1. What are the key OPPTS problems this research effort will address?

A major need within OPPTS are tools that can be used to prioritize and rank large chemical lists, and estimate information where data gaps exist. ASTER (ASsessment Tools for the Evaluation of Risk), integrates the aquatic component of the ECOTOX database and a quantitative structure activity relationship (QSAR) based expert system. ASTER is designed to provide high quality data for discrete chemicals by reporting measured toxicity values in the associated databases when available and QSAR-based estimates when data are lacking, with estimates of uncertainty and notification when information is too uncertain to make a prediction.

The ECOTOX database and ASTER system contribute to APMs in both LTG1 and LTG2 in the Safe Pesticide, Safe Products MYP. Under LTG2 this research effort will identify data gaps associated with interspecies correlation models, and will facilitate the identification of structural analogs and associated toxicity information to estimate potential hazard of untested chemicals or chemicals with limited toxic effects information.

2. What is the proposed research approach?

Under this research effort, NHEERL will be collaborating with OPP/EFED on the release of ASTER to EPA's Intranet. Models within ASTER will be upgraded and the system capabilities will be expanded, including the capability to rank large lists of chemicals based on data available in ASTER (e.g., ecotoxicity, environmental partitioning, environmental persistence, and chemical bioconcentration in tissues), and searching the ECOTOX database for structural analogs.

3. How does this research support the conceptual model for addressing this LTG?

The updating and improvements to the ASTER system will facilitate the identification of structural analogs and associated toxicity information to estimate potential hazard of untested chemicals or chemicals with limited toxic effects information.

4. If this project is successful, what products or tools will result from the effort?

Interim deliverables will include, a beta-version of the sub-structure search version of ECOTOX for testing by OPP/EFED in December of 2005, and a beta-version of the ranking module in January of 2007. These products will assist EFED in ranking and prioritization efforts and estimation of toxicity and physical / chemical parameters where data gaps exist, thereby improving risk assessments.

APM. Implement a sub-structure search feature within the ECOTOX database	FY07
APM. Release of ASTER via the EPA Intranet	FY07
APM. Release of ASTER Ranking Module	FY08

Project 3: Simulating Metabolism to Enhance Effects Modeling

1. What are the key OPPTS problems this research effort will address?

For OPPTS chemicals that lack adequate data on toxicological and exposure potential (e.g., certain PMN and HPV chemicals; inert pesticide ingredients; anti-microbial pesticides), the challenge is to create the means to efficiently and credibly predict toxic potency. A major uncertainty that has long been recognized in evaluating chemical toxicity is accounting for metabolic activation of chemicals resulting in increased toxicity. The proposed research will develop a capability for forecasting the metabolism of xenobiotic chemicals of EPA interest, to allow prediction of the most likely chemical metabolites to be formed. The information is interfaced with toxic effect models allowing prediction of parent chemical toxic potential and of chemical metabolites of equal or greater toxicity than the parent chemical. These predictions will enable reasonable decisions to be made as to whether or not empirical studies are required to further refine a risk assessment, and will allow OPPTS to move toward a new assessment paradigm that targets specific effects data needs, for specific chemicals and exposures, that is essential to assess and manage risks appropriately.

2. What is the proposed research approach?

An existing metabolism simulator will be refined to focus on metabolic transformations most likely to increase toxic potential for an endpoint under study in a related project, i.e., chemical estrogenicity (refer to Project1. A. 1). Metabolic transformations types shown to enhance estrogenicity (e.g., ring hydroxylation and O-dealkylation reactions resulting in hydroxylated products) but which are currently under-represented in an existing simulator will be studied using chemicals selected from priority OPP lists. For selected chemicals, metabolic pathways will be determined using rat (and/or fish) *in vitro* metabolism systems. Analytical methods will be developed and used to verify bioactivated metabolites formed in each system under study. Chemicals will additionally be tested in metabolically-competent liver slices from male fish. Upon exposed to chemical, metabolites are measured, and the production of vitellogenin mRNA will be determined. Vitellogenin is an egg yolk precursor protein not normally produced in male fish but highly inducible upon exposure to chemicals that bind and activate the ER. Chemical binding to fish ER will also be verified for chemicals and their putative bioactivated metabolites (when available) in an associated project (refer to Project A.1.A.). Newly generated metabolic maps will be used to re-train the metabolism simulator and thus increase reliability and predictivity.

3. How does this research support the conceptual model for addressing this LTG?

The research helps in better understanding toxicity pathways from initiating event to response for metabolically activated chemicals. A computational tool is provided that allows prediction and prioritization of chemicals for which measured data is lacking.

4. If this project is successful, what products or tools will result from the effort?

A simulator of metabolism, optimized for reaction types leading to enhanced ER binding will be provided, with a demonstration of how a metabolic simulator is efficiently enhanced for endpoints of concern.

APM. Develop experimental and analytical methods for in vitro chemical metabolism studies with emphasis on reaction types relevant to enhanced toxicity.	FY06
APM. Report on approach to evaluate and enhance metabolism simulator performance through incorporation of metabolic maps for reaction types and chemicals on EPA priority lists	FY07

Approach B: Methods

Project 1: Alternative Methods for Screening and Prioritization of Developmental Neurotoxicants

1. What are the key OPPTS problems this research effort will address?

The need to evaluate the potential effects posed by thousands of chemicals is a major challenge confronting EPA. Many chemicals lack data on basic toxicological potential (e.g., PMN and HPV chemicals, inerts), and current testing approaches require extensive resources. The area of developmental neurotoxicity testing has been identified in particular. The problem for OPPTS is that the current developmental neurotoxicity testing (DNT guideline) is costly, time consuming, and uses large numbers of animals. OPPTS needs efficient test methods which can be used to screen and prioritize chemicals for their potential to induce developmental neurotoxicity.

2. What is the proposed research approach?

This research will develop in vitro cell culture models of the key events in brain development and develop methods to measure behavioral, morphological and neurochemical outcomes in a limited number of non-mammalian species. Assays of developmental endpoints will be optimized for quantitative analysis using high-throughput technologies. The predictive capability of the in vitro and non-mammalian test batteries will be assessed using a training set of known developmental neurotoxicants. This set of chemicals will be composed of compounds from different chemical classes that are "generally regarded as developmental neurotoxicants" and will be linked to the DNT database being developed in Project A.1.A.

3. How does this research support the conceptual model for addressing this LTG?

A screening approach using cell-based models and alternative species addresses the need to delineate a strong linkage between responses observed at lower and higher levels of biological organization. This research addresses several questions associated with developing a first tier approach for screening for developmental toxicity including: 1) are there predictive biomarkers of key events in the developing nervous system that can be assessed in vitro; 2) how do we apply technological advances in high-throughput testing, genomics, and/or proteomics to develop rapid in vitro screening assays; 3) is there a homologous, non-mammalian model of neurodevelopment that can be used as a rapid screen for developmental neurotoxicity.

4. If this project is successful, what products or tools will result from the effort?

These high-throughput assays, will provide OPPTS with a screening battery for a first tier ranking of developmental neurotoxicity and prioritize them for further testing. The database that results from data generated by testing the set of known developmental neurotoxicants using high-throughput methods will provide OPPTS with information on the utility and limits of the screening battery, and provide guidance for the interpretation and potential use of data from these alternative methods in a risk assessment context.

APM. Report on quantitative assays of neurodevelopmental endpoints for cell-based and non-mammalian models	FY08
APM. Report on predictive ability of test battery for developmental neurotoxicity	FY10

Project 2: Toxicity Pathway-Specific Protein Expression Models for Chemical Screening and Prioritization

1. What are the key OPPTS problems this research effort will address?

Current resource and data requirements necessary to evaluate the large number of chemicals mandated under TSCA, FIFRA and FQPA has created the impetus to evolve from extensive hazard testing toward a targeted, hypothesis-based approach for chemical risk assessments. To achieve this operational shift, OPPTS requires toxicity pathway-based information which links credible diagnostic markers with adverse growth, reproductive and developmental outcomes. Additionally, an understanding of the conservation of predictive markers across vertebrate classes capable of distinguishing specific chemical categories is necessary for development of species extrapolation models.

2. What is the proposed research approach?

Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF MS) is used to examine protein expression profiling as a means to screen chemicals for their mode of action. In vitro exposure of fish hepatocytes and short-term in vivo minnow exposures are used to link diagnostic expression profiles between tissue level and whole organism assays, and across multiple fish species. Culture media (in vitro) or plasma (in vivo) samples from control and exposed treatments are applied to Protein Chip arrays to produce protein expression profiles unique to each treatment. A binary classification model is constructed from control and treatment protein profiles to identify differentially expressed proteins predictive of the mode of action of interest. As proof of concept, research efforts are initially focused on developing diagnostic models with established estrogenic and androgenic chemicals because of their well characterized mode of action.

3. How does this research support the conceptual model for addressing this LTG?

Protein expression profiling addresses the LTG1 intermediate-term need for targeted in vivo tests based on MOA and targeted omics-based in vitro screens. Profiling the differential expression of proteins associated with established toxicological pathways, and linking these profiles across multiple levels of biological organization provides OPPTS with a powerful predictive tool for screening and prioritization of chemicals. This research addresses OPPTS needs for increased efficiency and effectiveness of testing programs while providing information necessary to estimate the toxicological potential of a chemical or chemical class to elicit an adverse outcome.

4. If this project is successful, what products or tools will result from the effort?

This research will provide a proof-of-concept for using protein expression profiling in one species as a means to screen chemicals for pathway specific toxicity. This approach could be customized to screen HPV chemicals, inerts and anti-microbial pesticides of interest to OPPTS. Protein profiling could also be used to develop an "omics"-based approach to understanding differences in species sensitivity to chemical categories, and incorporated into existing in vitro and short-term in vivo assays needed to support hypothesis-based risk assessment and regulatory decisions.

APM. Report on protein expression recognition models to predict chemical mode of action.	FY06
APM. Report on multilaboratory validation of protein expression models to predict chemical mode of action.	FY07
APM. Report on cross-species protein expression recognition models predictive of chemical mode of action.	FY08

Long Term Goal 2: To create the scientific foundation for probabilistic risk assessment methods to protect natural populations of birds, fish and other wildlife

Issue

The research proposed under LTG 2 of this plan responds to OPPTS' needs in two specific problem areas. First, OPPTS needs efficient methods, including models, to review, register, and regulate thousands of chemicals in a timely fashion. Second, the Scientific Advisory Panel for the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) specifically recommended that the Office of Pesticide Programs (OPP) conduct probabilistic assessments of risks to ecosystems associated with pesticide use. Foremost among the Panel's recommendations was the value of moving beyond the current single-point deterministic assessment approach toward the development of the tools and methodologies necessary for a probabilistic assessment of risk. As a follow-up to this and other reviews, the Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM) was formed by OPP to develop specific recommendations for revising the assessment process. Once ECOFRAM provided their recommendations, which were peer reviewed, a Refined Risk Assessment Team (RRA Team) was formed to develop an implementation plan to incorporate probabilistic tools and methods into the ecological risk assessment process for pesticides. In response to OPP's strategic direction in moving toward probabilistic assessments, NHEERL developed a Wildlife Research Strategy (WRS, US EPA 2004) which describes a tiered approach using a series of wildlife risk assessments. Such assessments can range from most general and broadly based (screening level) to most realistic, accurate and situation-specific (definitive level). Probabilistic methods can be used for any of these approaches. Throughout all tiers, the response of wildlife[1] populations is the assessment endpoint of concern. The appropriate final tier for a wildlife risk assessment is based on a risk management analysis that weighs risk assessment uncertainties against the context of the management decision and the costs associated with a 'wrong' decision compared to the costs of gathering and employing increasingly realistic and accurate data and models.

The response of wildlife populations is the assessment endpoint of concern.

The conceptual model for NHEERL's WRS describes this process for probabilistic ecological risk assessment as four critical steps representing exposure and effects components (Figure 5). Note that this would correspond to the Tier IV, fully probabilistic model described by the ECOFRAM report, i.e., assessing both exposure and effects metrics within a probabilistic framework. The first step involves the spatial and temporal characterization of stressors; e.g.,

[1] "Wildlife" is defined to mean all vertebrate animal species, in both terrestrial and aquatic systems.

contaminant exposure, habitat suitability, and introduced species, that may adversely impact the wildlife population of concern. Based on the results of step one, quantitative chemical dose-response relationships and habitat-response relationships at the individual level are developed in step two (e.g., relationships to fecundity and life-stage specific probability of survival). In steps three and four, these demographic rates are used in population models to generate outputs describing population growth rates or other appropriate population-level endpoints. Spatially explicit models may be used to determine cumulative population dynamics across the landscape and assess relative effects due to chemical exposure as well as other forms of habitat disturbance. Using this process, it is possible to make projections about how wildlife populations may be impacted by many stressors other than pesticides or contaminants that result directly or indirectly from human activities (e.g., habitat loss and alterations, introduced species, hunting pressure). In addition, because neither stressors nor wildlife populations are distributed uniformly within the environment, the interplay between spatial and temporal heterogeneity in wildlife population structure and spatial and temporal patterns of stressors is a major factor controlling the severity of effects on wildlife populations. Thus, a critical feature of this research is the development of probabilistic models that deal explicitly with the spatial distribution of populations and stressors over time. While steps provide general guidelines for population-level risk assessment, the level of accuracy and realism appropriate for each step varies with assessment needs and management goals. For example when applied in the context of site-specific risk assessment, these models can be applied to real landscapes, by interfacing with geographical information systems (GIS). For more generalized regional or national-level assessments (e.g., for pesticide registration), simulated

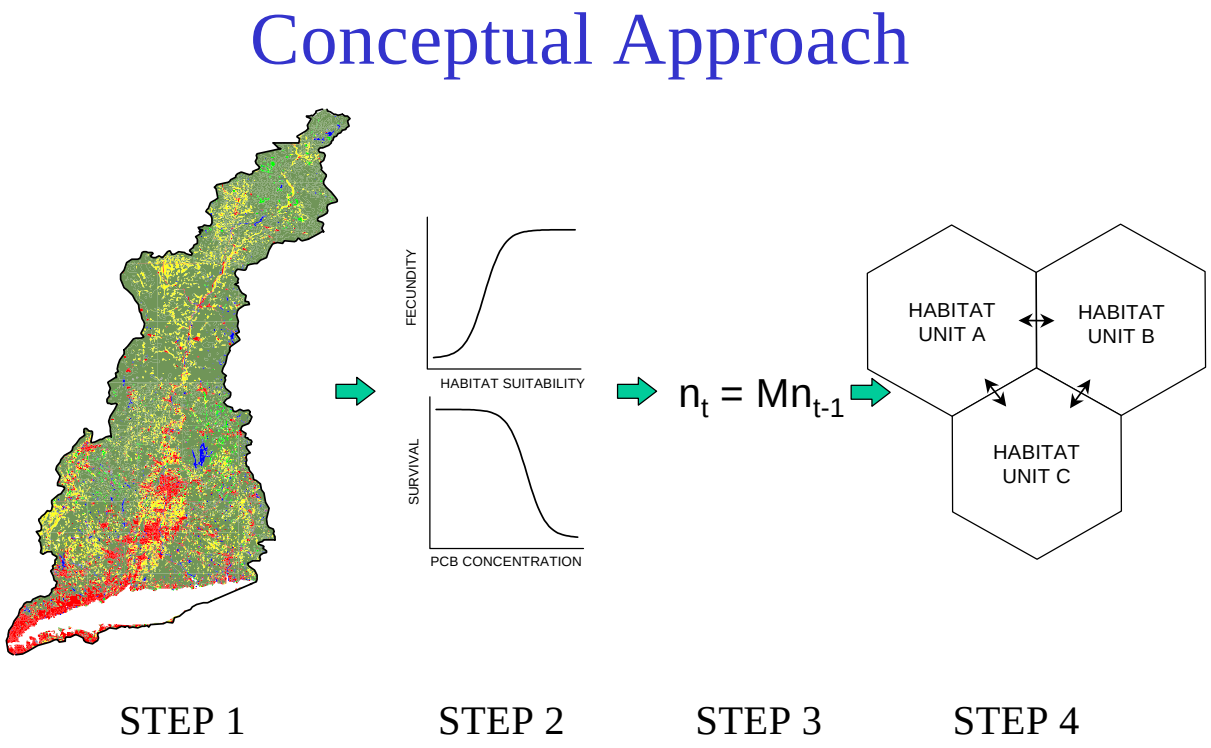

Figure 5. Conceptual approach probabilistic ecological risk assessment

(or constructed) landscapes can be used that mimic the general characteristics of the ecosystems of concern.

Problems, Products and Impact

OPP is leading the way in expanding ecological risk assessments (ERAs) to provide probabilistic expressions of risk to aquatic and terrestrial wildlife populations, including reducing uncertainties in all tiers of the risk assessment process as uncertainties that are extrapolated from limited data sets are better defined and put into context. For this purpose, methods are required to support population-level ERAs :

a) of increasing degrees of specificity, detail and realism
b) to determine the absolute /or relative (incremental) risk of chemical and non-chemical stressors
c) at varying geographical regions/ or other areas of regulatory concern.

Data needs for each assessment differ depending upon the goals of the assessment and the desired level of confidence in the outcome. For example, screening level or "lower tier" assessments may involve a relatively simple evaluation of chemical fate and effects, including qualitative judgments about the likelihood of exposure and potential for bioaccumulation. In the case of compounds regulated under the pre-manufacturing notification (PMN) process, this may involve expert opinion about which class the chemical belongs to and selection of an appropriate analog or quantitative structure-activity relationship (QSAR). A similar approach may be applicable to some "inert" ingredients of pesticide formulations, antimicrobials and High Production Volume (HPV) chemicals. More involved assessments are required for "active" compounds covered by FIFRA as well as some TSCA chemicals of special concern. These "higher tier" assessments are more likely to include quantitative evaluations of effects and exposure information and may involve model-based efforts to extrapolate data from surrogate species and chemicals. Currently, data available for these needs also differ considerably. Chemicals with pesticidal properties fall under the jurisdiction of FIFRA Subdivision E, Parts 158.145 and 158.150. For these substances, registrants are required to provide a core set of data regarding toxicity to wildlife and vegetation. This information may be used to prohibit the registration of a pesticide, or more frequently, to regulate its use, including the timing, method and rate of application. There is also concern about the toxicity of "inert" ingredients that are combined with active compounds in formulated products (i.e., in the final commercially-produced pesticide). In contrast, under the TSCA Section 5 PMN review process, importers or manufacturers of chemicals are not required to submit any effects data: the EPA can request toxicity test results, e.g., using mammalian (usually in support of human health questions), avian or aquatic species only if there is reason to believe that a specific adverse effect can occur. Generally, the only reported result is a single acute value, e.g., the mammalian oral LD50, which for the sake of the ecological risk assessment is taken to be representative of all wildlife. Potential adverse environmental effects of existing chemicals are assessed under TSCA Section 4 (e.g., High Production Volume program) or TSCA Section 7 (e.g., imminent hazards, e.g., lead, perfluorinated compounds, and perchlorate). These may include both

LTG 2 will 1) make better use of current OPPTS data , and 2) move the science forward through the inclusion of more sophisticated data and analyses

detailed terrestrial and aquatic risk assessments, depending upon use or disposal patterns.

To address these needs, NHEERL's scientific research will be directed toward improving current risk assessment processes used by OPP and OPPT for new and existing chemical risk assessments, with specific focus on their needs to 1) make better use of the current data that they receive during product registration or for existing substance review, and 2) move the science forward through the inclusion of more sophisticated data and analyses.

To address the needs of OPP, OPPT and other program offices, NHEERL's WRS describes critical research that will provide the scientific foundation for probabilistic risk assessments to inform decisions related to protection of natural populations of birds, fish and other wildlife. Specifically, this research focuses on the development of those approaches and tools whose advancement is most needed to conduct spatially-explicit, population-level risk assessments. Furthermore, the conceptual model (Figure 5) provides a critical path for research to develop these integrated components of ecological risk assessment. NHEERL's research within LTG2 focuses on the latter three of the four critical steps, which must integrate with the first step (exposure characterization) to complete this process. These latter three steps include the development of methods to improve the characterization of effects of chemical and non-chemical stressors on the fitness of individuals of various species, on the viability of populations of species with varying life histories, and on the dynamics of spatially-structured populations inhabiting heterogenous landscapes. Research described in this plan is organized into these three critical areas, and is intended to reduce currently recognized uncertainties, and lay the foundation to address emerging issues and needs.

To address the needs of OPP, OPPT and other program offices, NHEERL's Wildlife Research Strategy describes critical research that will provide the scientific foundation for probabilistic risk assessments to inform decisions related to protection of natural populations of birds, fish and other wildlife

Science Questions

Consistent with the goals of NHEERL's WRS, research will be conducted to address the following scientific questions associated with tiered, probabilistic population-level risk assessments for aquatic and terrestrial wildlife species:

a) Can we develop approaches to extrapolate toxicological data across species, media, and individual-level response endpoints?
b) Can we develop approaches to predict population-level responses to stressors? Can we identify those responses at the individual level that have the greatest influence on population-level responses?
c) Can we develop approaches to evaluate relative risks from chemical and non-chemical stressors on spatially structured terrestrial wildlife populations across large areas, regions, or other areas of regulatory concern?

Program Project Areas

The goal of the research that NHEERL will conduct under LTG 2 is to develop scientifically valid approaches to assess risks to wildlife populations from multiple stressors. This requires a means of mathematically integrating dose-response and habitat suitability relationships as well as computer platform for site-specific, spatially-explicit population modeling. To address these needs,

NHEERL will provide scientific support for OPPTS that focuses on three program project areas:

1. Methods to extrapolate available information to species, biological endpoints and exposure scenarios of concern, including methods to characterize wildlife species most vulnerable to specific chemical exposures (**Program Project Area 3**)
2. Methods to predict the impact of stressors on population dynamics of species, and groups of species with similar life history strategies (**LTG 2 Program Project Area 4**)
3. Methods to predict the impact on wildlife populations of interacting stressors in complex, spatially-defined habitats (**LTG 2 Program Project Area 5**)

Descriptions of these key research areas, and specific research areas follow below. The research projects are summarized in Table 4. Finally, important research areas are identified that are not to be addressed in NHEERL's near-term research implementation plans (gap areas).

More detailed descriptions of these projects are found in Appendix B on the NHEERL SP2 website: http://www.nheerl.epa.gov/nheerl_science/pesticide_implementation/

Table 4. Summary of NHEERL LTG 2 Program Projects

Approach	Title	Partners	Contact/Lead
Program Project Area 3: Species/Endpoint Extrapolation Methods			
A: Empirical Data and Interspecies Extrapolation Models	Project 1. ECOTOX Database	NHEERL/MED; NHEERL/GED; OPP/EFED	C. Russom, MED
	Project 2. Interspecies Extrapolation Models	NHEERL/GED	M. Barron, GED
B. Mechanistically Based Approaches to Evaluate Differences in Species Sensitivity	Project 1. In Vitro and in Vivo Biotrans-formation in Fish: Implications for Physiologically Based Toxicokinetic Models	NHEERL/MED	J. Nichols, MED
	Project 2. In Vitro Mode-of Action Models to Predict Differences in Toxicity among Species	NHEERL/NTD; NHEERL/MED	T. Shafer, NTD
C. Extrapolation Methods for Under-Represented Taxa, Lifestages, Chemicals and Endpoints	Project 1. Short-term Test Endpoints to Predict Multi-Generational Effects for Aquatic Species	NHEERL/MED	R. Johnson, MED
	Project 2. Extrapolating Across Endpoints and Lifestages	NHEERL/MED; NERL/EERD-Cinn	G. Ankley, MED
Program Project Area 4: Population Extrapolation Methods			
A. Simple Screening Tools to Project Population Responses	Project 1. Incorporating Simple Population Models Into EFED Risk Assessment Process	NHEERL/AED	T. Gleason, AED
	Project 2. Development of Methods for Managing Demographic Data	NHEERL/MED	M. Etterson, MED

B. Incorporate More Realism (Complexity) Into Projections of Population Responses	Project 1. Incorporating Stochasticity Into Demographic Models	NHEERL/AED; NHEERL/GED	J. Grear, AED S. Raimondo, GED
	Project 2. Incorporating Density Dependence Into Demographic Models	NHEERL/AED; NHEERL/GED	J. Grear, AED S. Raimondo, GED
	Project 3. Genetic Influences on Population Dynamics	NHEERL/AED; NERL/EERD-Cinn	D. Nacci, AED
Program Project Area 5: Spatially-Explicit Population Models			
A: Avian Wildlife	Project 1. GIS avian species databases	NHEERL/WED	N. Schumaker, WED
	Project 2. PATCH Model	NHEERL/WED	N. Schumaker, WED
	Project 3. PATCH Demonstration Project	NHEERL/WED; NHEERL/MED	N. Schumaker, WED R. Bennett, MED
	Project 4. PATCH Case Study	NHEERL/WED	N. Schumaker, WED L.Nagy WED
	Project 5. PATCH Model Selection	NHEERL/WED; NHEERL/MED	L.Nagy WED M. Etterson, MED
B: Aquatic Species	Project 1. Spatially-explicit Models for Aquatic Species	NHEERL/GED	M. Baron, GED S. Raimondo, GED

Program Project Area 3: Species/Endpoint Extrapolation Methods

Approach A: Empirical Data and Interspecies Extrapolation Models

Project 1: ECOTOX Database

1. What are the key OPPTS problems this research effort will address?

OPPTS requires access to information on the effects of chemicals on wildlife species in order to efficiently assess the ecological risks of a myriad of chemical compounds. ECOTOX serves this need by providing a comprehensive web-based system, maintained by ORD, that provides information on chemical effects to ecological species. ECOTOX is also linked to the ASTER (Assessment Tools for the Evaluation of Risk) expert system, which provides physical/chemical property information, environmental fate, biodegradation, and toxic effects for aquatic species. ASTER invokes structure-activity based models to estimate the parameter when empirical data are not available in the associated databases. ECOTOS is critical to OPP's risk assessment process for all species (including actions under the Endangered Species Act) and is used to supplement the data required by FIFRA. The ECOTOX database and ASTER system contribute to APMs in both LTG1 and LTG2 in the Safe Pesticide, Safe Products MYP. Under LTG1 this research effort provides a library of QSARs for prediction of aquatic toxicity endpoints when measured data are not available, facilitates chemical prioritization and ranking needs, sets a framework for incorporation of additional toxicity pathway-based QSARs, and provides a tool for sub-structure searching of the ECOTOX toxic effects databases.

2. What is the proposed research approach?

Under this research effort, literature acquisition and encoding for the ECOTOX database will focus on EFED priorities (e.g., studies on pesticides undergoing re-registration meeting data quality requirements for use in final risk assessments.) NHEERL/MED will work with NHEERL/GED on format requirements for the early-life stage data for use in the models to be developed under the 2007 APM.

3. How does this research support the conceptual model for addressing this LTG?

The ECOTOX system facilitates LTG2 efforts by identifying data gaps associated with interspecies correlation models including the release of previously unpublished chronic test data for organic chemicals, and will facilitate the identification of structural analogs and associated toxicity information to estimate potential hazard of untested chemicals or chemicals with limited toxic effects information.

4. If this project is successful, what products or tools will result from the effort?

Interim deliverables will include database summaries of data of relevance to the EFED risk assessments. Discussions with OPP/EFED and NHEERL/GED will be ongoing to ensure that all relevant information on the test result are available in a usable format. The final product will be summary of data of relevance to the EFED risk assessments and the early-life stage tests. These products will assist EFED in identifying reliable toxic effects information, and thereby improve the final risk assessment.

APM. Report on results from early-life stage tests with the fathead minnow on a series of industrial organic chemicals	FY 06

Project 2: Interspecies Extrapolation Models

1. What are the key OPPTS problems this research effort will address?

For ecological risk assessments conducted in support of the registration of chemicals, OPPTS requires predictive toxicological models that maximize the usefulness of limited information and minimal data sets to protect a broad array of aquatic and wildlife species, including endangered species and other species that have not been tested or are not feasible to test. To address data gaps in species sensitivity, the Interspecies Correlation Estimation (ICE) program has been developed to predict acute toxicity to under- represented taxa, and the Acute to Chronic Estimation (ACE) program has been developed to predict chronic toxicity.

2. What is the proposed research approach?

The proposed research will continue development and evaluation of the ICE and ACE toxicity estimation programs. Key improvements will include: 1) identification and expansion of appropriate surrogate species; 2) increased representation of industrial organic and inorganic chemicals; and, 3) incorporation of chemical classes and chemical mode of action categories. Additional research will evaluate sources of uncertainty in ICE and ACE outputs, including: a) evaluation in uncertainty and protectiveness for chronic mortality versus other endpoints such as growth and reproduction; b) incorporation of mode-of-action categories into correlation models; and c) approaches for reducing variability in toxicity predictions for birds and mammals.

Existing databases used for extrapolating under represented species, chemicals, endpoints, and life stages will be expanded by including additional wildlife and aquatic species toxicity data that have not yet been compiled. Both ICE and ACE will be validated by comparing model predictions to measured values. ICE acute toxicity estimates will be compared to measured LC50 values for multiple species, chemical classes, and modes of action. ACE chronic mortality estimates will be compared to measured values for no effect and low effects on chronic survival, growth and reproduction endpoints determined in available chronic toxicity tests. The results of the validation and software refinement will be incorporated into new user guidance to allow determination of the reliability of both ICE and ACE estimates.

3. How does this research support the conceptual model for addressing this LTG?

The ICE/ACE modeling approaches contributes importantly to the ability to predict the effect of chemicals on aquatic and wildlife species, an essential component of the conceptual model for ecological risk assessment (Figure 5). Specifically, this approach facilitates the estimation of potential hazard for untested chemicals or chemicals with limited toxic effects information.

4. If this project is successful, what products or tools will result from the effort?

This project will provide OPPTS with predictive toxicological models through computer software available on CD or the internet through web-based programs. New user guidance, expanded data sets, and refined and improved tool functionality will also be expected to be useful to Office of Water and other program offices involved in ecological risk assessment.

APM. Evaluation of model uncertainty and identification of critical data gaps in ICE model extrapolation to under-represented taxa	FY 06
APM. Establishment of a database for organic chemicals for inclusion and refinement of interspecies correlation model	FY 07
APM. Provide revised and validated ICE and ACE toxicity estimation tools	FY 08

Approach B: Mechanistically Based Approaches to Evaluate Differences in Species Sensitivity

Project 1: In Vitro and In Vivo Biotransformation in Fish: Implications for Physiologically Based Toxicokiinetic Models

1. What are the key OPPTS problems this research effort will address?

OPPTS needs information on the effects of a myriad of chemicals on a broad range of aquatic and wildlife species, most of which cannot be acquired directly. Consistent with the tiered ecological risk assessment process, OPPTS requires predictive toxicological models to extrapolate effects across species and chemical categories. These models vary in specificity, accuracy and information requirements. Higher tier risk assessments may require mechanistically-based models to extrapolate toxicity information among chemicals, species and lifestages. Physiologically based toxicokinetic (PBTK) models provide an important predictive tool for tier three risk assessments, where extrapolations based on detailed studies of well-known chemicals and species are appropriate.

2. What is the proposed research approach?

This project consists of three interrelated efforts that advance the development and application of PBTK models for compounds that undergo metabolic biotransformation in aquatic species. Initially, an effort will be made to synthesize available *in vitro* fish metabolism data and scale up this information for incorporation into PBTK models. In a second effort, experimental work will be conducted to test the accuracy of model predictions based on *in vitro* data by making *in vivo* metabolism measurements for selected compounds. Finally, a new experimental system for high-throughput collection of metabolism information will be developed, along with a mathematical model that translates this information into *in vivo* metabolic rate and affinity estimates.

3. How does this research support the conceptual model for addressing this LTG?

The PBTK modeling approach contributes importantly to the ability to predict the effects of chemicals on aquatic and wildlife species. This research addresses several questions that limit current efforts to extrapolate toxicity data among species, including: 1) how well does *in vitro* data predict *in vivo* rates of metabolism; 2) how variable are metabolism rates within and among species; 3) under what circumstances does metabolism impact chemical bioaccumulation; 4) what type of metabolism data has the most utility for incorporation into PBTK models.

4. If this project is successful, what products or tools will result from the effort?

This research supports OPPTS's need for the development of PBTK models predictive of chemical toxicity in fish, and quantitative methods to translate existing *in vitro* rate data into estimates of *in vivo* metabolism (which may be incorporated into PBTK models). In addition, this research will provide new methods to collect metabolism information in a more rapid and cost-effective manner.

APM Development of a database framework of biochemical, physiological and anatomical parameters to support physiologically based species extrapolation models	FY 05
APM: Develop quantitative model to estimate *in vivo* metabolic rates for fish from microdialysis data	FY 06
APM: Report on the utility of *in vitro* metabolic assays to predict *in vivo* metabolism in fish.	FY 08

Project 2: *In Vitro* Mode-of Action Models to Predict Differences in Toxicity among Species

1. What is/are the key OPPTS problem(s) that this research effort will address?

For ecological risk assessments conducted in support of the registration of chemicals, OPPTS requires predictive toxicological models that maximize the usefulness of limited information and minimal data sets to protect a broad array of aquatic and wildlife species, including endangered species and other species that have not been tested or are not feasible to test. For some well–studied classes of chemicals (such as pesticides action on ion channels), mode-of-action (MOA) models provide useful approaches to predict toxicity. Furthermore, *in vitro* methods provide important alternatives to *in vivo* and whole animal methods. However, these approaches should be further explored and validated to provide OPPTS with tools to identify sensitive species and extrapolate chemical effects across species.

2. What is the proposed research approach?

To examine potential toxicodynamic differences among species, a pilot project will be initiated to determine the relative differences in insecticide activity at known sites of action in the nervous system. This will provide a test of concept that *in vitro* measurements of effects via a known mode-of-action can predict species differences in toxicity. Initially, voltage-sensitive sodium channels will be selected as an endpoint, and the pyrethroids will be utilized as a class of compounds for comparisons. Both the insecticidal and acute toxicological effects of pyrethroids are mediated via voltage-sensitive sodium channels, and these channels have been cloned from insects, rodents and humans. These will serve as starting points for cross species comparisons by obtaining these clones, expressing them in *Xenopus* oocytes, and measuring pyrethroid effects on their function using electrophysiological techniques. The ability to predict toxicity will be determined by comparison of potency on ion channel function to LD/LC50 values among the different species.

3. How does this research support the conceptual model for addressing this LTG?

This research is integrated with ongoing research in the Human Health Implementation plan as well as related to cross-species work (animal-to-human) that has been conducted under the Air Toxics research plan. This research is targeted towards providing improved methodologies for predicting and identifying sensitive populations, by reducing the need to test all chemicals and to employ animals in toxicity testing.

4. **If this project is successful, what products or tools will result from the effort?**

If successful, *in vitro* assays can be used to predict species sensitivity over a broad range of classes, without use of *in vivo* testing or animal models. *In vitro* assays developed from this approach could be developed into high-throughput screens, which could be used to test the species sensitivity of a large number of compounds. Screens based on other receptors could provide the basis for databases and/or computational models of species sensitivity.

APM. Report on the utility of *in vitro* mode of action-based assays to predict species differences in susceptibility	FY 10

Approach C: Extrapolation Methods for Under-Represented Taxa, Lifestages, Chemicals and Endpoints

Project 1: Short-term Test Endpoints to Predict Multi-Generational Effects for Aquatic Species

1. What is/are the key OPPTS problem(s) that this research effort will address?

In enforcing its legislative mandates, OPPTS needs to assess the potential effects of chemicals on populations of fish species. In response to concerns for the possible adverse effects of chemicals on sublethal effects, emphasis has been placed recently on identifying chemicals that adversely impact reproduction. To date, the primary research emphasis as been on the development of short-term screening assays, and predictive models based on the data derived from these short-term screening assays. While these assays and models reduce costs and animal numbers used in testing, their relevance based on reproductive success must be determined. As chemicals are screened for their ability to elicit biomarker responses linked to reproduction, the implications of these effects on fish and wildlife populations must be evaluated. Specifically, endpoints measured on individuals following short-term exposures must be evaluated for their relevance to adverse outcomes associated with long-term multi-generational exposures, and linked to population-level effects.

2. What is the proposed research approach?

Substantial progress has been made internationally toward the development of medaka short-term and multi-generation test protocols. These tests will be used to evaluate the relationship between effects observed in shorter-term tests to consequences and effects observed in longer-term tests. Specifically, a harmonized medaka (*Oryzias latipes*) multi-generation exposure protocol will be used to evaluate the following population-relevant endpoints; fecundity, fertility reproductive behavior, phenotypic and genotypic sex of each generation. These data will be used to link *in vitro* and short-term *in vivo* tests to population-relevant endpoints. Some measures such as genotypic/phenotypic sex ratios will be assessed across several generations. To provide a generalized assessment of chemical effects, representative chemicals with known discrete toxicity pathways will be selected for testing.

3. How does this research support the conceptual model for addressing this LTG?

This research will result directly in improved methods to acquire information describing the quantitative relationship between chemical concentrations and adverse biological effects, which may translate into ecological, population-level effects.

4. If this project is successful, what products or tools will result from the effort?

This research is coordinated with research identified under the Endocrine Disrupting Compounds MYIP, designed to provide a short-term in vivo medaka assay that can be used to characterize linkages between reproductive endpoints and diagnostic biomarkers for endocrine disrupting chemicals. Here, the results of short- and long-term tests using medaka will be used to characterize the utility and limitations of short-term tests in predicting population effects for classes of chemicals known to affect reproductive pathways.

APM. Peer-reviewed guidance document for performing histopathology on gonadal tissues from small fish exposed to endocrine disruptors	FY06
APM. Develop a medaka protocol for assessing transgenerational effects of reproductive toxicants on the HPG axis	FY07

Project 2: Extrapolating Across Endpoints and Lifestages

1. What is/are the key OPPTS problem(s) that this research effort will address?

The Environmental Fate and Effects Division (EFED) The Office of Pesticides (OPP) within OPPTS requires methods and models to conduct probabilistic risk assessments that incorporate consideration of mechanism-specific data in the context of population-level effects. Some molecular level responses serve as useful indicators of modes/mechanisms of action (MOA) for specific categories of toxic chemicals. However, their value as predictive indicators of population-level effects requires further validation.

2. What is the proposed research approach?

This work will use a combination of whole organism endpoints, "omic" approaches, and population modeling to (a) identify molecular biomarkers of exposure to chemicals representing several MOAs and (b) link those biomarkers to effects that are relevant for both diagnostic and predictive risk assessments. Biomarkers mechanistically-related to specific MOAs of concern within the hypothalamic-pituitary-gonadal (HPG) axis will be identified using genomic and/or proteomic approaches with the zebrafish, a useful model from the standpoint genomic analysis, and/or the fathead minnow, the small fish model most commonly used by the Agency for both lab testing and field monitoring. A subset of chemicals representative of these different MOA will be extensively characterized using a short-term reproduction assay with the fathead minnow. These results will provide input for population modeling and provide crucial information in terms of understanding the consequences of changes in gene and/or protein expression with respect to apical responses. This research will be conducted through collaboration with an interdisciplinary network of EPA (MED, NHEERL; EERD, NERL-Cincinnati) and non-EPA partners (e.g., University of Florida, Joint Genome Institute of the Department of Defense).

3. How does this research support the conceptual model for addressing this LTG?

This work directly addresses the scientific challenge of linking changes in gene and protein expression (i.e., responses reflective of MOA) to alterations at higher levels of biological organization including cellular-, tissue-, and organism-level responses. Ultimately, this information will be incorporated into population models that will support both diagnostic and predictive risk assessments.

4. If this project is successful, what products or tools will result from this effort?

The research tools provided by this research will directly support the needs of OPPTS Office of Science Council and Policy for a fathead minnow model test system (in the US and, under the auspices of the Organization for Economic Cooperation and Development (OECD), throughout the world). EFED's need for a small fish toxicological model will also be served. In addition, this research is highly integrated with ORD's research in Endocrine Disruptors, and co-funded by the National Center for Computational Toxicology.

APM. Development of a technique based on toxicogenomic/proteomic approaches as a bases for extrapolating across species and biological levels of organization	FY07

Program Project Area 4: Population Extrapolation Methods

Approach A: Simple Screening Tools to Project Population Responses

Project 1: Incorporating Simple Population Models Into EFED Risk Assessment Process

1. What is/are the key OPPTS problem(s) that this research effort will address?

The OPPTS\OPP\EFED was challenged by the ECOFRAM report, of the mid 1990s, to redesign their ecological risk assessment tools to make their risk assessments more ecologically relevant. In support of this need, scientists from NHEERL's ecology divisions are working with EFED to implement the approaches identified in the NHEERL Wildlife Research Strategy in support of the risk assessment needs of EFED. This specific effort is aimed at incorporating population models into the EFED risk assessment process. By incorporating population level analyses in their risk assessment process, EFED will be taking a significant step forward in terms of making their risk assessment process more ecologically relevant.

2. What is the proposed research approach?

This effort will explicitly incorporate relatively simple screening matrix population models into the EFED risk assessment process and represents an important first step in moving from concept to implementation. Close coordination with scientists in EFED will be an essential component to this effort. This effort will involve refining population models that have been developed for EFED and integrating these models with the exposure and effects models currently used by EFED. Some of the factors for consideration include the software code and how to make a seamless connection between our population models and EFEDs existing risk assessment models; translating EFED effects endpoints into stressor response models that can be used in the population models; data quality for population model parameter estimates; model interpretation including decisions on which population model endpoints will are most appropriate for assessing risks; as well as some level of training and continuing technical support.

3. How does this research support the conceptual model for addressing this LTG?

This effort is directly aligned with the conceptual model for incorporating population level analysis into the EFED risk assessment process. In fact, this effort begins the important process of moving from conceptual models of addressing population response to actual implementation of population level analyses.

4. If successful, what products or tools will result from this effort?

This effort will represent the first step towards actual implementation of population modeling approaches into the risk assessment process. It is envisioned that in roughly one year, we would participate in a Science Advisor Panel (SAP) review of the EFED risk assessment model including the incorporation of screening level population models. Successful completion of this step would allow EFED to begin using population level analyses in their risk assessment process and take concrete steps towards meeting the challenges of increasing the ecological relevance of their risk assessment process as identified in the ECOFRAM report.

APM. Provide a catalogue of simple deterministic population models for terrestrial and aquatic species of concern	FY06

Project 2: Development of Methods for Managing Demographic Data

1. What is/are the key OPPTS problem(s) that this research effort will address?

Information on demographic parameters, such as survival, fecundity (i.e., reproduction), immigration and emigration rates, for species of concern is critical for developing population models for incorporation into OPPTS's ecological risk assessment process. These parameters are primarily gleaned from published literature and are vulnerable to numerous sources of bias and uncertainty, which are often either explained in the original publication or may be inferred based on the assumptions and mathematics used. To understand the usefulness of population models for accurately characterizing risks in ecological risk assessments, it is important to understand the nature and relative importance of these sources of bias and uncertainty. This understanding is important in determining if the quality of the population model is sufficient to answer the risk management question.

2. What is the proposed research approach?

This research is developing a methodology for evaluating the reliability of predictions made using population projection models that rely on published demographic parameters. To calculate these parameters from field data, researchers make a suite of assumptions about the data being used, and about the existence and structure of ecological mechanisms operating in the population. Often, the assumptions made during the parameter estimation phase contradict assumptions made during the projection phase, and this is likely to increase error and uncertainty in the resulting population projections. We are using computer simulations that mimic the population-level risk assessment process at three important phases-- data collection, parameter estimation, and population projection– to attempt to characterize the risk-assessment scenarios that result in the most serious errors and to provide a set of diagnostic criteria that may be used to identify when problems in the quality of population projections may be present.

3. How does this research support the conceptual model for addressing this LTG?

This research supports the conceptual approach and goal of the NHEERL Wildlife Research Strategy to develop scientifically valid approaches for assessing risks to wildlife populations from multiple stressors. Specifically, this research is focused on assuring that the uses of population modeling in risk assessment are commensurate with the quality of data used in developing population models.

4. If this project is successful, what products or tools will result from this effort?

NHEERL will provide guidance on how to design a population projection that takes into account the quality of the available demographic data and the information available from model selection methods applied to the original data. This also would become an important foundation for assessing what level of inference that may be drawn from a population model conditional upon the quality of the input data to the model. This guidance will aid in the development of screening level population models (see project 1 above).

APM. Development of methods for assessing the quality of published demographic parameters for use in population-level risk assessments	FY07

Approach B: Incorporate more realism (complexity) into projections of population responses

Project 1: Incorporating stochasticity into demographic models

1. What is/are the key OPPTS problem(s) that this research effort will address?

OPPTS needs methods to support probabilistic ecological risk assessments. Incorporating stochasticity into demographic models will expand the suite of population-level endpoints from deterministic estimates of population growth and elasticity to probabilistic assessments of risk of population decline or quasi-extinction, and will more explicitly address higher tier ecological risk assessment needs of OPPTS.

2. What is the proposed research approach?

NHEERL proposes research to explore methods to incorporate stochasticity into population modeling. NHEERL's research will provide guidance about the use of measured variance from both laboratory and field studies and the probability density functions used to represent them, and will evaluate assumptions about correlations between vital rates in time-varying stochastic matrix models. In addition to testing these models with simulated data, NHEERL will compare results with results from other modeling approaches. In particular, methods involving the use of diffusion approximations of population count data to estimate risk metrics will be researched and compared with matrix model approaches. The primary rationale for examining count-based methods is the relative ease with which count data can be obtained, and the apparent but still untested promise of using citizen-collected wildlife count data. Additional research is proposed using a specific application for which data from laboratory as well as field data are available to parameterize and test models. Specifically, a basic stochastic model will be developed for the mysid shrimp *Americamysis bahia* subjected to simulated pesticide exposure regimes. Field collected population abundance data will be used to identify natural variability of *A. bahia*, which will be related to laboratory derived matrix models of mysids exposed to various pesticides. The result of this synthesis is a more realistic projection of mysid populations exposed to various toxicants in the field.

3. How does this research support the conceptual model for addressing this LTG?

This research will improve approaches for assessing risks to wildlife populations from multiple stressors by providing guidance on choosing the appropriate stochastic modeling approaches specific data and assessment scenarios.

4. If this project is successful, what products or tools will result from this effort?

This project will provide methods to incorporate variation into population modeling projections using species and data of interest to OPPTS. In addition, guidance on the development and interpretation of stochastic projections using toxicity test data will be provided along with application examples.

APM. Provide stochastic population modeling approaches for risk assessments of aquatic populations	FY07
APM. Provide stochastic population modeling approaches for risk assessments of avian populations	FY08

Project 2: Incorporating Density Dependence Into Demographic Models

1. What is/are the key OPPTS problem(s) that this research effort will address?

The OPPTS\OPP\EFED was challenged by the ECOFRAM report, of the mid 1990s, to redesign their ecological risk assessment tools to make their risk assessments more ecologically relevant. In response to this need, simple, deterministic density-independent population projection models are being developed and incorporated into the risk assessment process. While such approaches are appropriate for generic or screening assessments, higher tiered risk assessments require approaches that incorporate greater realism and complexity. Specifically, OPPTS needs methods that permit the incorporation of density dependence into projections of population responses to chemical stressors.

2. What is the proposed research approach?

This research will examine the ways in which various forms of density dependence affect assessment endpoints. For example, a number of data scenarios will be generated using stochastic simulation models that are parameterized to represent the range of scenarios encountered in risk assessment. Examples of axes along which these scenarios will vary include life history traits such as longevity and fecundity, intrinsic growth rate, strength of density effects, and the functional form of the underlying density dependence (e.g., Ricker, Beverton-Holt, etc). The consequences of model misspecification will then be examined in terms of bias and uncertainty in assessment endpoints. Those density dependence formulations with the greatest flexibility and adaptability to assessment scenarios will be incorporated into EPA's existing suite of population modeling tools along with guidelines for their selection and use.

3. How does this research support the conceptual model for addressing this LTG?

This research will improve approaches for assessing risks to wildlife populations from multiple stressors by incorporating density dependence formulations into risk assessment population models. A more central focus of this research, however, will be to guide the selection and application of these density dependent models for specific data and assessment scenarios.

4. If this project is successful, what products or tools will result from this effort?

This project will result in a set of population models that use alternative methods for incorporating density dependence and will provide practical guidance materials for choosing between these models. This guidance will include tutorial examples of model choice for given data scenarios, parameterization and application of models, and interpretation of assessment endpoints.

APM. Guidance on incorporating density dependence into population models for populations and species of concern	FY07
APM. Report on the implications to population risk assessment of population density-stressor interactions	FY09

Project 3: Predicting Stressor Impacts on Populations /Population Genetics

1. What is/are the key OPPTS problem(s) that this research effort will address?

While simple or generic approaches are appropriate for screening level ecological risk assessments, higher tiered risk assessments require approaches that incorporate greater realism and complexity. Specifically, OPPTS needs methods that can incorporate the effects of chemical stressors on population genetic structure into projections of long-term population viability. Models and methods integrating population genetics and dynamics, while currently lacking, have the potential to improve the accuracy of population modeling projections of the effects of chemical stressors, and therefore increase their value in the ecological risk assessment process.

2. What is the proposed research approach?

The proposed research will review current methods and models to integrate population genetics and dynamics, and evaluate their potential usefulness to project chemical effects. This evaluation will include consideration of data requirements, i.e., the accessibility of molecular genetic information, and data utility, i.e., the potential for genetic information (as gene functions become known) to inform population dynamics models. Selected experimental and simulation models will be adapted and implemented to refine and test hypotheses associated with chemical risks and genetic perturbations to population persistence. Results from these systems will provide specific examples of the (data) costs and consequences to projections of incorporating genetics into population dynamic models. Results will also be used more generally to provide guidance relative to current methods and propose solutions to current limitations that will enhance appropriate inclusion of genetic information to estimate effects on population persistence of chemical stressors.

3. How does this research support the conceptual model for addressing this LTG?

This research supports the conceptual approach and goal of the NHEERL Wildlife Research Strategy to develop scientifically valid approaches for assessing risks to wildlife populations from multiple stressors. Specifically, this research is focused on providing better understanding and guidance on the need for and uses of more complex population modeling approaches as tools for integrating and projecting effects of stressors on wildlife populations.

4. If this project is successful, what products or tools will result from this effort?

This research will result in guidance on the development of population models that take into account genetic qualities of populations: structure diversity and conditional fitness correlates. This guidance will aid in the development of definitive level population models.

APM. Review published methods and models to incorporate genetics into population viability models	FY07
APM. Report on methods to incorporate genetics into population projections of stressor effects	FY09

Program Project Area 5: Spatially-Explicit Population Models

Approach A: Avian Wildlife

Project 1: GIS Avian Species Databases

1. What are the key OPPTS problems this research effort will address?

The use of spatially explicit population models requires geographically-referenced data as input, and even simple matrix models rely on accurate demographic information for species of interest. This project will augment a recently constructed web-accessible database that is coupled to a geographical information system (GIS). This allows end-users (i.e., anyone within the Agency with access to the EPA Intranet) to query the data for underlying GIS information as well as space-based avian demographics and agricultural statistics. Appropriate simulations can then be developed to explore potential risks of pesticides to bird populations under a variety of different cropping practices and environmental stressors.

2. What is the proposed research approach?

This work will build on the web-based system being developed for herbicide risk assessments under LTG 4 (this Plan). Data will reside on an EPA server and be available to Agency clients via ARC-IMS, an ESRI product that allows end-users to query maps and spatial data using their web browser, thus eliminating the need for additional software or knowledge of GIS methodology. Data will be obtained through collaborative efforts with federal, state and local agencies, or purchased as necessary. All databases will be referenced to counties, which will be the common spatial unit for analyses. Data layers will include (but likely not be limited to): political boundaries (states, counties); human population census data; major geomorphic attributes; crop locations (both conventional and genetically engineered); resident plants (i.e., noncrop species); bird distributions (from Breeding Bird Survey data); pesticide use (location and type of registered pesticides); and wind speed. Additional layers can be added as requested by OPP or Regional offices.

3. How does this research support the conceptual model for addressing this LTG?

Long Term Goal 2 is intended "to create the scientific foundation for probabilistic risk assessment methods to protect natural populations of birds, fish, and other wildlife" (italics added). Spatially explicit population models are necessary to develop adequate assessments and, by extension, georeferenced data are required to run the models.

4. If this project is successful, what products or tools will result from the effort, and how would OPPTS use them?

Enhancement of a web-accessible database of information on crop practices and locations that is being developed for EFED under LTG 4 of this plan by including avian demographic information. Additional layers developed for other purposes include native plant species in the U.S. and wind speed. The basic structure of the database has been completed; avian demographics and pesticide use information needs to be developed and input to the database (A web-accessible, GIS with information on cropping practices, pesticide use, and avian demographics. Database/presentation to OPP, APM: FY2007).

APM. A web-accessible, GIS with information on cropping practices, pesticide use, and avian demographics	07

Project 2: PATCH model

1. What are the key OPPTS problems this research effort will address?

EPA's Office of Pesticide Programs (OPP) has been challenged to develop risk assessment tools that operate at increasing levels of biological organization, specifically populations. Current methods address only the fate of individual organisms exposed to a single stress, viz. pesticides. The development of a spatially explicit wildlife population model will provide a tool for scaling from individuals up to populations in a manner that addresses the complexities of real landscapes, and that evaluates the cumulative effects of pesticide use and other factors such as habitat alteration and environmental variability. The model and associated, datasets, documentation, and example analyses being developed are designed for use by OPP scientists and managers but are sufficiently generalizable to be applicable to OPPTS and other Program Office needs.

2. What is the proposed research approach?

Several spatial population modeling techniques currently are available and represent the range of analysis tools currently available for use in a tiered risk assessment framework. The research being conducted here will enhance the PATCH model (Program to Assist in Tracking Critical Habitat) and tie it specifically to pesticide issues. PATCH is a spatially-explicit, individual based life history simulator that incorporates GIS representations of real or hypothetical landscapes. This work will result in a significantly upgraded model that will allow users to define the organism's life history and stressor regimes at run-time.

3. How does this research support the conceptual model for addressing this LTG?

Long Term Goal 2 is intended "to create the scientific foundation for probabilistic risk assessment methods to protect natural populations of birds, fish, and other wildlife" (italics added). Natural wildlife populations inhabit real landscapes and are thus exposed to varying habitat structure and quality, as well as an array of human-caused stressors. Predicting the consequences to a real population of one perturbation (e.g. a pesticide application) without considering other human activities and changing environmental conditions would be unrealistic, and does not represent the best available scientific methodology. This research will produce a general state-of-the-art model accessible and usable by all EPA risk assessors that is not limited to any specific landscape, species, or suite of stressors.

4. If this project is successful, what products or tools will result from the effort?

OPP staff will be trained by ORD scientists to use the increasingly sophisticated versions of PATCH (initial versions are already available on the web), as well as a web-accessible database coupled to a geographical information system. Documentation and example analyses also will be provided on the web, These tools are being designed specifically for OPPTS, and OPPTS scientists and managers for use in probabilistic risk assessments addressing potential long-term impacts of pesticide use on wildlife populations, with particular application for evaluation of threatened and endangered species.

APM. Delivery of PATCH version with interfaces to distributed databases and population models	06
APM. PATCH II framework version (Windows) with generalized life history module, general stressor module, and GIS accessibility	08

Project 3: Patch Model – Agricultural Demonstration

1. What are the key OPPTS problems this research effort will address?

EPA's Office of Pesticide Programs (OPP) has been challenged to develop risk assessment tools that operate at increasing levels of biological organization, most importantly at the scale of wildlife populations. But wildlife inhabit complex landscapes and are exposed to multiple interacting stressors including environmental variability, habitat degradation, and a range of human activities. A shift in focus towards wildlife populations must therefore be accompanied by an increase in model complexity. Just how much additional model complexity is required is a question of enormous practical importance to OPP. Simpler assessment tools are easier to parameterize and use, but are less able to integrate the range of stressors that impinge upon populations. The use of overly simplistic models increases the danger of incorrectly concluding there will be "no population-level effect" of a pesticide (a Type II error). This concern is of paramount importance in the case of threatened and endangered wildlife populations exposed to pesticide applications. This research will examine the degree of model complexity necessary to avoid committing such Type II assessment errors. We will also explore how the requisite model complexity varies with landscape configuration, wildlife species life history, and stressor regime.

2. What is the proposed research approach?

The PATCH model will be used to simulate wildlife population responses to pesticide application within agricultural landscapes. Multiple landscape configurations, wildlife life histories, and stressor regimes will be explored. Subsequent analysis will elucidate the minimum level of model complexity necessary to avoid committing a Type II error.

3. How does this research support the conceptual model for addressing this LTG?

Long Term Goal 2 is intended "to create the scientific foundation for probabilistic risk assessment methods to protect natural populations of birds, fish, and other wildlife" (italics added). Wildlife populations inhabit real landscapes and are thus exposed to varying habitat structure and quality, as well as an array of human-caused stressors. This demonstration study will help the Program Office evaluate the relative importance of landscape structure, stressor interactions, and the amount and pattern of pesticide use on bird populations which will further their understanding of the effort needed to make predictions with required degree of accuracy.

4. If this project is successful, what products or tools will result from the effort, and how would OPPTS use them?

The understanding of OPP staff about how birds interact with their environments will be significantly enhanced. ORD will provide OPP staff with a demonstration of changing population projections, through interactive presentations of PATCH model simulations. A scientific publication will provide a peer-reviewed document in support of such discussions.

Demonstration of importance of spatial complexity of pesticide avian risk estimates: Report/presentation to OPP	06

Project 4: Patch Case Study

1. What are the key OPPTS problems this research effort will address?

EPA's Office of Pesticide Programs (OPP) has been challenged to develop risk assessment tools that operate at increasing levels of biological organization, specifically populations. Current methods address only the fate of individual organisms exposed to a single stress, viz. pesticides. The development of a spatially explicit wildlife population model will provide a tool for scaling from individuals up to populations in a manner that addresses the complexities of real landscapes, and that evaluates the cumulative effects of pesticide use and other factors such as habitat alteration and environmental variability. This case study is designed to illustrate how life history of species of interest enhance model realism and the importance of landscape structure on populations exposed to pesticides.

2. What is the proposed research approach?

A 3-year study of the western bluebird will be conducted in the Willamette Valley, OR, encompassing multiple habitat types (e.g., tree farms, grass seed farms, residential and natural areas) to develop information about importance of habitat selection and movement patterns on the realism of the PATCH model simulations. Further information about interactive effects of stressors (e.g., pesticides and nest parasites) as well as indirect effects of pesticides (e.g., reduced food supply) also will aid in understanding long-term impacts of pesticide use.

3. How does this research support the conceptual model for addressing this LTG?

Long Term Goal 2 is intended "to create the scientific foundation for probabilistic risk assessment methods to protect natural populations of birds, fish, and other wildlife" (italics added). Natural wildlife populations inhabit real landscapes and are thus exposed to varying habitat structure and quality, as well as an array of human-caused stressors. Predicting the consequences to a real population of one perturbation (e.g. a pesticide application) without considering other human activities and changing environmental conditions is unrealistic, and does not represent the best available scientific methodology. This case study will provide further documentation about relative importance of stressor interactions and indirect effects of pesticides on avian habitat.

4. If this project is successful, what products or tools will result from the effort, and how would OPPTS use them?

OPP staff will be provided access to databases of information of avian demographics. These case studies will help train OPP scientists to select appropriate parameters for use in matrix models and/or spatial simulators when assessing risks of pesticides in real agroecosystems. This will be particularly useful for assessing risks to threatened and endangered species whose populations are already under stress from factors other than pesticides.

Project 5: PATCH Model Selection

1. What are the key OPPTS problems this research effort will address?

EPA's Office of Pesticide Programs (OPP) has been challenged to develop tools to predict risks to avian populations. There are numerous population models available or under development that range in complexity from simple 2-stage matrix models to highly complex, individually based, spatially explicit, bioenergetic approaches. Each model has its own strengths and weaknesses, depending upon the question being asked, the degree of accuracy/precision required, data availability, and intrinsic factors of differing avian life histories. Each risk assessment is also unique, and in order to minimize both Type II errors (predictions that there will be no effect of a pesticide on a population when, in fact, there will be) and the effort required to reach a risk-based decision, it is critical to select the model that best suits the analysis being conducted. The work described here will develop the theoretical underpinnings and suggest a framework to optimize model selection.

2. What is the proposed research approach?

A series of hierarchical population simulations will be conducted to optimize model selection based on available data quality and model specificity. Our most realistic analytic tool (the PATCH model) captures the influence on a population of landscape structure, environmental variability, and multiple interacting stressors (e.g. pesticide use, crop harvesting, etc.). We will use PATCH to approximate reality, and compare its predictions to those obtained from a suite of models designed to capture successively less landscape structure, life history details, and species-stressor interactions. These alternative models, ranging from nested matrix models, to, matrix models with environmental stochasticity, to deterministic 2-stage matrix models, will explore when model output fails to simulate "reality" with required precision. The effect of variable amounts of uncertainty in input data will be examined as well using a reverse falsification approach to find the population - habitat dynamics that put birds most at risk. It will address a) What is the minimum model complexity that you need? and b) What is the minimum data set you need to have faith in the results?

3. How does this research support the conceptual model for addressing this LTG?

Long Term Goal 2 is intended "to create the scientific foundation for probabilistic risk assessment methods to protect natural populations of birds, fish, and other wildlife" (italics added). Several types of population models are being developed under this Research Plan to support this objective. The work described here provides necessary information for selecting which model is most useful (and cost/time effective) for addressing particular risk-related questions.

4. If this project is successful, what products or tools will result from the effort, and how would OPPTS use them?

This work will result in "rules of thumb" for selecting a model depending upon the risk question being addressed and the amount and quantity of data available. It will inform OPP of which types of data are more important in increasing model accuracy (e.g., toxicity data vs. life history data vs. landscape/environmental stochasticity information), which will help guide future information needs.

Optimizing model complexity: applications in avian population risk assessment	06

Approach B: Aquatic species

Project 1: Spatially-Explicit Modeling for Aquatic Species

1. What are the key OPPTS problems this research effort will address?

Pesticide runoff off can occur over large areas on the landscape scale, potentially impacting hundreds of miles of river or coast line. A recent NOAA report (Pait et al., 1992) describes the Gulf of Mexico region as having both the highest application rate of agricultural pesticides and the highest hazard-normalized pesticide application in the U.S. Gulf of Mexico estuaries are among the most productive ecosystems on earth and serve as nurseries for 80-90% of the region's commercial and recreational fish and shellfish. The potential for fish species to be exposed to agricultural pesticides in these estuaries indicates the need to critically assess their population-level responses on a spatially explicit basis.

2. What is the proposed research approach?

A spatially explicit fish population model will be developed, and evaluated using the sheepshead minnow (*Cyprinodon variegatus*), or similar species. Pesticide data from an extensive literature base will be used to initially develop demographic population models, which can then be expanded into spatially-explicit models using literature-based information on natural history, native habitat, distribution, range, and occurrence of the species within the Gulf of Mexico. Additionally, spatiotemporal data collected as part of the Environmental Assessment and Monitoring Program (EMAP) will provide valuable realism in developing spatial models. Modeling approaches, such metapopulation and reaction-diffusion models, may be applied to project the population-level effects in spatially explicit terms and develop an approach that is intended to have broader applicability to other species, stressors, and coastal areas.

3. How does this research support the conceptual model for addressing this LTG?

Spatially explicit population models are necessary to develop adequate assessments because 1) agricultural pesticides are seldom traceable to a point source, 2) agricultural pesticides are present at the landscape scale and may impact large areas of Gulf of Mexico estuaries, 3) tides and currents result in the diffusion of agricultural pesticides through the estuaries, 4) habitat resources used by fishes in Gulf of Mexico estuaries are not uniformly distributed, and 5) different life stages of many fish species occupy different niches, resulting in habitat-dependent exposures.

4. If this project is successful, what products or tools will result from the effort, and how would OPPTS use them?

This research will serve as a proof of concept on the application of spatially explicit fish population modeling to the assessment of large scale pesticide exposure and risks in coastal areas, significantly enhancing the understanding of OPP staff about how coastal fish in the Gulf of Mexico are potentially affected by pesticides. ORD will provide OPP staff with a demonstration of changing population projections, through interactive presentations of model simulations. A scientific publication will provide a supporting peer-reviewed document.

Develop spatially-explicit population models to predict effects of stressors on fish populations	08
Report on spatially-explicit population models for coastal fish	10

Long Term Goal 3. Evaluating Potential Risks Associated with Biotechnology Products

[Note: The material in this section was abstracted from "Biotechnology Research Program". EPA 600/R-03/068. U.S. Environmental Protection Agency, March 2005.]

Background

Biotechnology presents a wealth of opportunities to improve crop productivity, nutritional value, and resistance to pests and other stresses. However, there are potential risks to human health, natural ecological systems, and existing agricultural systems that need to be evaluated so that the products of biotechnology can be properly regulated. Currently, EPA regulates several biotechnology products (e.g., pesticides, either produced by plants or by microorganisms, and non-pesticidal substances such as industrial enzymes, biosensors, and bioremediation agents produced using microorganisms). While discussions continue about whether EPA's scope of regulation should be broadened to include animals (e.g., insects) that produce pesticidal substances and plants and/or animals that produce non-pesticidal substances, no such products are currently under review by EPA.

From a human-health perspective, a major concern is the potential toxicity and allergenicity associated with genetically modified foods. Potential adverse effects can arise from intended modifications (i.e., from the pesticidal substance) or from unintended effects resulting from the production of an unexpected metabolite. To date, the products approved by EPA for use in human food have all been proteins that degrade rapidly and from which no chronic effects would be expected. This approach has been accepted by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel. However, some members of the public have raised the concern that proteins not previously part of the food supply could be allergens. It is, however, well accepted that the genetic material itself will not cause an acute or chronic toxic effect; thus DNA has been exempted from tolerance.

The regulation of biotechnology products is also intended to minimize the risks to human-managed (i.e., agricultural) and natural ecosystems. Such risks are associated with the consequences of unintended release of genetically modified plants or their bioengineered genes. For example, there are concerns about the ecological impacts resulting from replication and persistence of transformed organisms that could out-compete native species in a given environment. In terms of the risks to agricultural systems, there are potentially adverse long-term consequences of evolved resistance to the biotechnology product. Pest resistance could render related conventional products (e.g., the spores of *Bacillus thurigiensis* [Bt] used as pesticidal sprays) ineffective, reducing crop productivity or necessitating increased usage of conventional pesticidal applications (which also threatens ecosystem health).

Goal

The goal of the Biotechnology Research Program is to provide the scientific information needed to assess and manage the risks of biotechnology. The research program will accomplish this by providing the tools needed to generate information about biotechnology products, by generating the

knowledge needed to understand the nature and magnitude of potential risks and benefits resulting from the use of biotechnology products in commerce, and by providing the means to prevent or control such risks. At this time the focus is on the risk from plant incorporated protectants (PIPs).

Research Approach

The Agency Challenge

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) evaluates the environmental risks posed by pesticides and chemicals to safeguard all Americans, including children and other vulnerable members of the population, as well as our most threatened species and ecosystems. Within OPPTS, the Office of Pesticide Programs (OPP) regulates the use of all pesticides in the United States and establishes maximum levels for pesticide residues including genetically engineered pesticides. While it is not anticipated that biotechnology products will pose new types of risks, these new products are often on the cutting edge of science and regulatory policy; and research is needed to ensure that their safety can be appropriately evaluated. Also, within OPPTS, the Office of Pollution Prevention and Toxics (OPPT) regulates the use of industrial chemicals and certain biotechnology products, such as microorganisms used in the manufacture of specialty chemicals and bioremediation agents. OPPT also implements the Pollution Prevention Act and hence has an interest in biotechnology product stewardship that would lead to "green" chemicals. OPPT has an emerging interest in certain transgenic plants for uses such as phytoremediation and enhanced wood production although OPPT does not implement regulatory oversight in this area at this time. Currently, most biotechnology risk assessment research concerns in OPPTS are affiliated with pesticidal products.

In assessing safety, the basic framework for pesticide regulation provides guidance as to the nature of any new risks. EPA has recognized that PIPs (or genetically engineered plants which produce their own pesticides) represent potentially different risks from traditional, chemical pesticides. For example, while there is very low worker exposure and no spray drift, there are issues regarding gene flow to wild relatives and pollen movement spreading the new pesticides to non-altered crops. In addition, the level of protein produced is very small; but, because proteins can be allergens and even low levels of a new protein might lead to sensitization and eventual allergic reactions, special emphasis on allergenicity is given to evaluation of these products.

Because proteins can be allergens and even low levels of a new protein might lead to sensitization and eventual allergic reactions, special emphasis on allergenicity is given to evaluation of genetically engineered plants which produce their own pesticides

With respect to environmental risk, effective tools and methods are needed to minimize the likelihood of negative ecological effects such as the following:

Ecosystems

a) harm to non-target species, such as soil organisms, non-pest insects, birds, and other animals;
b) disruptive effects on specific biotic communities;
c) irreparable loss of changes in species diversity and genetic diversity within species.

Agri-Systems

a) creating new or more vigorous pests and pathogens;
b) exacerbating the effects of existing pests through hybridization with related transgenic plants or microorganisms.

Both

a) pleiotropic or epistastic effects on plant physiology due to emerging metabolic engineering approaches. [These manipulations, found in current commercialized transgenic organisms, may result in unintended effects in host plants or non-target plants that may inadvertently receive the transgene.];
b) rapid development of resistance to the engineered crop by target pests that may result in greater use of more harmful pesticide products over the long term.

With respect to protecting human health, EPA must assess whether pesticides derived through biotechnology are at least as safe as their conventional counterparts; and the EPA must ascertain that any levels of additional or unique risk are clearly defined. A significant challenge may occur in the future if transgenic technology results in more substantial and complex changes in exposures and/or if such technology results in compounds that are more toxic. It is important to note that many pesticidal substances such as phenols and aldehydes occur naturally in plants.

Progress also needs to be made in developing definitive methods for the identification and characterization of protein allergens. EPA needs to be able to estimate accurately the levels of exposure to the genetically engineered products that are released in the environment, and EPA needs the means to evaluate whether such exposures are potentially harmful. Finally, EPA needs to find and evaluate ways to prevent or mitigate identified risks.[2] Contributing to the effectiveness of this program are the integration of science activities across the risk assessment paradigm and strong interaction with OPPTS. The proposal also includes the use of workshops involving scientists with appropriate expertise from academia, industry, and other government institutes. These workshops are designed to develop broad consensus with respect to research needs and strategies and to coordinate research efforts not just within EPA, but with the National Institutes of Health (NIH), the Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), and the scientific community at large.

Key Science Issues with Respect to Biotechnology Products

A problem-directed research program has been developed that focuses on five key issues:

1) the potential allergenicity of proteins introduced into the food supply by gene transfer;
2) the potential ecological effects of biotechnology products on non-target species;
3) the spread of transgenes to the natural environment via seed dispersal or gene flow to sexually compatible relatives;
4) the development of pesticide resistance in the target species; and
5) strategies for identifying the risks of concern and effective risk management technologies to

[2]Under both FIFRA and TSCA, EPA also has an obligation to consider the potential benefits of biotechnology pesticide products.

mitigate these risks when monitoring studies indicate unintended adverse consequences are likely to occur.

Performance Results and Expected Benefits

By FY 2008, this research program will result in an improved capability to assess the risks of allergenicity from GM food, improve the capability to assess the ecological risks associated with genetically modified organisms (GMOs), and develop tools to understand and better manage gene transfer and resistance. Program performance will be measured in the following ways:

a) the use of research products by OPPTS in the registration and re-registration process, both in hazard identification and risk assessment, and in setting risk management requirements for registration;
b) general acceptance of these methods by other regulatory agencies; and
c) public acceptance of EPA's approach to regulating GM crops.

Program Project Areas

ORD's research to meet OPPTS' needs is conducted all of the Laboratories and Centers. *Only NHEERL's portion of the research program will be described in this plan*. It focuses on:

a) the potential allergenicity of proteins introduced into the food supply by gene transfer;
b) the spread of transgenes to the natural environment via seed dispersal or gene flow to sexually compatible relatives

A focus of NHEERL's research is the spread of transgenes to the natural environment via seed dispersal or gene flow to sexually compatible relatives

Research Approaches and research projects that will be conducted under LTG 3 are summarized in Table 5.

See "Biotechnology Research Program". EPA 600/R-03/068. U.S. Environmental Protection Agency, March 2005 for a detailed description of the entire program. This document can be found on the NHEERL SP2 website:

http://www.nheerl.epa.gov/nheerl_science/pesticide_implementation/

Table 5. Summary of NHEERL LTG 3 Program Projects

Program Project Area 6: Potential Allergenicity of Proteins Introduced Into the Food Supply By Gene Transfer			
Approach	Title	Partners	Contact/Lead
A: Develop Methods to Assess Dietary Allergenicity	Project 1. Potential Allergenicity of Genetically Modified Organisms	NHEERL/WED NCEA	M. Selgrade ETD
Program Project Area 7: Escape of Altered Plants to the Natural Environment and the Likelihood and Effects of Gene Transfer			

A. Evaluate the ability of some crops to transfer introduced genes through hybridization to wild and/or weedy relatives	Project 1. Evaluating Gene Flow from Genetically Modified Crops and its Potential Ecological Effects	NHEERL/ETD NCEA, NRMRL, NERL	L. Watrud, WED
	Project 2. Develop methods to evaluate transgenic plants and microorganisms for negative fitness effects of the transgene, and to evaluate whether environmental conditions or common stressors influence this process	NHEERL/ETD NCEA, NRMRL, NERL	L. Watrud, WED

Program Project Area 6: Potential Allergenicity of Proteins Introduced Into the Food Supply By Gene Transfer

Approach A: Develop Methods to Assess Dietary Allergenicity

Project 1: Potential Allergenicity of Genetically Modified Organisms

1. What is/are the key OPPTS problem(s) that this research effort will address?

The goal is to develop an animal model suitable for assessing potential allergenicity relative to other food proteins and for testing hypotheses regarding conditions (e.g., age, genetics) that contribute to susceptibility. This project focuses on the development of an animal model to predict allergenicity and will not address the need to monitor human populations.

Biotechnology presents a wealth of opportunities to genetically engineer crops to improve productivity, enhance resistance to pests and other stressors, and provide nutritional value. However, there is growing concern that there may be risks to human health that have not been adequately explored. The biggest concern is that, as a result of the introduction of novel proteins into the food supply, biotechnology may unwittingly introduce a potent food allergen that could seriously affect the health of susceptible individuals. Currently, there is no animal model that can be used to test proteins for potential allergenicity following oral exposure, nor are there other means to readily identify proteins that might be potent allergens. Furthermore, the mechanisms underlying the development of food allergy and the factors that contribute to individual susceptibility are poorly understood.

Models of allergenicity are needed to answer the following questions:

(a) Does dietary exposure to transgenic pesticide proteins induce immune, inflammatory, and histopathology responses typical of food allergy?
(b) Is the degree of digestibility inversely related to risk of allergenicity?
(c) Is early life the most vulnerable time for dietary allergy sensitization?
(d) Does the food matrix make a difference in allergic responsiveness?
(e) How potent is the transgenic pesticide protein in the induction of dietary allergic responses (i.e., what is the dose-response relationship relative to known food allergens)?
(f) Where there is potential for both respiratory and oral exposure, what are the risks when an individual sensitized by the respiratory route ingests the protein; and what are the risks of respiratory exposure in an individual sensitized by the oral route?

2. What is the proposed research approach?

The following critical path will be followed in order to address these questions:

(a) Develop a dietary allergy model in a laboratory rodent using a modification of the respiratory allergy protocols. Suckling, weanling, and adult rodents (BALB/c mice or Brown Norway rat) will be exposed by gavage or fed multiple times with various doses of a known food allergen to establish the ability to induce food-allergy responses. Allergic responsiveness will be judged based on the induction of antigen specific IgE and IgA in addition to gut mucosal eosinophil influx and respiratory responses. The lung and skin are the most frequent target organs even when the route of exposure is ingestion. Experimental conditions that most closely mimic food allergy in humans will be used in subsequent studies.
(b) Once the model is developed, rodents will be fed or gavaged multiple times with various doses of a prototype transgenic pesticide protein. The most likely candidate is the *Bt* toxin. Various forms of this toxin with varying degrees of digestibility will be tested. Allergic

responsiveness will be assessed based on results obtained from the above studies. Appropriate positive and negative controls will be incorporated into the model.

(c) Assess the responses to the transgenic pesticide protein allergen in both a purified form and in a food matrix. The food matrix is the way in which most human ingestion will occur, and it may provide an adjuvant effect. Therefore, exposure to the purified protein alone may not be adequate to assess its potential allergenicity. Using the model protocol, rodents will be gavaged with both the purified protein and an equivalent amount of the protein in a food matrix. Comparison of the responses should provide insight into the role of the food matrix in dietary allergy.

(d) Assess the relative potency of transgenic pesticide proteins when compared to known food allergens. Using the model protocol, responses to transgenic pesticide proteins will be compared to the responses of a range (strong to weak allergy inducers) of established food allergens.

(e) Use the model to assess effects of respiratory exposure following oral sensitization and oral exposure following respiratory sensitization.

3. How does this research support the conceptual model for addressing this LTG?

Long Term Goal 3 is intended to provide an improved capability to assess the risks of allergenicity from GM food. The work described here provides necessary information for selecting which model is most useful for assessing the risk of allergenicity from genetically modified food.

4. If this project is successful, what products or tools will result from the effort?

Models to predict dietary allergenicity from consuming crops containing PIPs and other biotechnology products will be developed and verified as a basis to assess the potential allergenicity of proteins introduced into the food supply by gene transfer

APM	Demonstrate the vulnerability of newborns/ identify windows of vulnerability.	8	ETD

Program Project Area 7: Escape of Altered Plants to the Natural Environment and the Likelihood and Effects of Gene Transfer

Approach A: Evaluate the ability of some crops to transfer introduced genes through hybridization to wild and/or weedy relatives

Project 1: Explore the factors influencing gene transfer rates to provide a basis for better assessments

1. What is/are the key OPPTS problem(s) that this research effort will address?

Currently, engineered crops are planted on tens of millions of acres in the U.S. alone. Pollen from transgenic crops may hybridize with related crops or weeds, potentially transferring the transgene to crop-crop or crop-weed hybrids. The resultant F1 hybrids may in turn self- or out-cross to other compatible species or may backcross to the transgenic or non-transgenic parent. In addition, the transgenic genes may move via feral plants or seeds; i.e., over-wintering transgenic plants or seeds that escape cultivation or via seeds that have fallen from planters, combines, trucks, or railroad cars during routine planting, harvesting, and shipping activities. Some transgenes may have a limited persistence due to their insert locations, characteristics of the genetic cassette, or the plants or microorganisms themselves. This could result in limiting exposure to the gene product. Methods are needed that can be used to predict the probability of gene flow occurring, the rate and extent of spread of the transgene, and identification of potential recipient species.

2. What is the proposed research approach?

Six major scientific questions will be addressed by this research:

(a) How far and with what frequency do transgenes move from GM crops into other plants?
(b) Which biological and non-biological factors affect gene flow?
(c) How long do transgenes persist in non-target host populations?
(d) How can the parameters identified in these studies be used in probabilistic risk assessment models?

In order to answer these questions, two lines of research will be conducted.

(a) Develop gene tracking methods – qualitative and quantitative molecular methods or other cytological, biochemical, or morphological markers will be developed to track transgenes (or components thereof) from GM crops to other crops or non-crop plants. Molecular methods (e.g., PCR) will be developed to facilitate detection of stress response at the genomic level in support of ecological effects studies.
(b) Select compatible crop/non-crop species – compatible plant species will be selected for greenhouse or field studies of gene transfer. Rates of transfer will be compared between novel and conventional crop protection genes to provide information on stability and persistence of genetic material. Differences due to life-history traits (e.g., pollination methods) will be assessed, as well as effects of type of genetic construct (e.g., nuclear vs. chromosomal inserts; single vs. multiple engineered traits; crop protection vs. crop nutrient quality traits; protein vs. non-protein metabolically engineered traits, etc.).

3. How does this research support the conceptual model for addressing this LTG?

Long Term Goal 3 is intended to provide an improved capability to better understand and manage gene transfer and persistence. The work described here provides necessary information to understand the factors influencing gene transfer rates from GM crops and informs the exposure analysis of an assessment of the ecological impact of gene transfer.

4. **If this project is successful, what products or tools will result from the effort?**

This research will result in guidelines or approaches for assessing rates of transfer of transgenes from crops to non-crop relatives and the likelihood that such genes will introgress and become a stable part of the recipient plants' genome(s). There are three specific outcomes for these studies:

1) an understanding of the potential for transfer of novel genetic material from GM crops to non-target plants and the associated ecological consequences of such exchange;
2) methods for determining and minimizing amounts and circumstances of gene transfer from proposed GM crops that can be provided by registrants when applying to OPP/BPPD for registration of new PIPs; and
3) identification of inputs for probabilistic risk assessment models of gene flow from GM crops.

APM	Methods for estimating frequency of gene transfer from GM crops to non-crop plants	6	WED
APM	Molecular methods (e.g., microarrays) applied to plant genomes for assessing genetic change and environmental stress	6	WED

Project 2: Develop methods to evaluate transgenic plants and microorganisms for negative fitness effects of the transgene, and to evaluate whether environmental conditions or common stressors influence this process

1. What is/are the key OPPTS problem(s) that this research effort will address?

While it is commonly argued that cultivated crops would not persist well outside of agronomic situations due to their need for high soil-fertility levels, little information is available on the survival, fertility, and out-crossing potential of hybrids formed between crops and compatible weedy or native species. Many species in each of the two latter categories (weedy and native species) commonly thrive in low fertility soils. It also is not known how exchange of transgenes will affect the overall fitness of non-crop plants, either enhancing or decreasing their ability to compete within the natural plant community. Methods are needed to allow such questions about ecological risks to be adequately addressed during development and deployment of crops with novel PIP transgenes.

2. What is the proposed research approach?

The following lines of research will be conducted: Evaluate ecological effects – studies will be conducted on the consequences of genetic transfer on fitness (i.e., survival, yield, biomass production) of recipient plants or on the transgenic crop plant itself (the latter case would be a model of potential for effects on nontarget plants). The potential for persistence of the gene through succeeding generations of the parental and other compatible species also will be studied. Differences in transgene insert locations, characteristics of the genetic cassette, or the species involved will be evaluated. Fitness consequences of gene expression will be studied in multispecies communities subjected to various environmental stressors. Molecular methods such as microarrays will be used to study genomic level stress responses in relation to fitness parameters. Develop probabilistic risk assessment models - risk assessment methods to evaluate potential adverse effects of gene transfer from GM crops will be developed using probabilistic methods building on similar work currently underway for conventional herbicides. This includes estimates of exposure (e.g., probability of gene transfer for a given crop location, environmental factors, etc.) and effects (e.g., probability of ecological effects as a consequence of novel genetic material moving into non-crop plant species).

3. How does this research support the conceptual model for addressing this LTG?

Long Term Goal 3 is intended to provide an improved capability to better understand and manage gene transfer and persistence. The work described here provides necessary information to understand the consequences and potential adverse fitness effects of gene transfer rates from GM crops. It informs the effects analysis of an assessment of risk of potential ecological impact of gene transfer.

4. If this project is successful, what products or tools will result from the effort?

This research will result in guidelines or approaches for determining frequency and magnitude of negative fitness effects resulting from transfer of transgenes from crops to non-crop relatives. This will lead to understanding associated ecological consequences of gene exchange and will provide inputs for probabilistic ecological risk assessment models of gene flow from GM crops.

APM	Ecological consequences of movement of transgenes from GM crops to non-crop plants	7	WED
APM	Probabilistic methods for assessing ecological risk of genetic transfer from GM crops	8	WED

Long Term Goal 4. Dealing with Novel or Newly Discovered Pesticide and Other Chemical Hazards

Introduction

Despite the rigorous registration testing and guidelines for adverse health and ecological effects of chemicals and pesticides, unanticipated issues emerge periodically that require EPA program offices, such as the Office of Prevention, Pesticides & Toxic Substances (OPPTS) to revisit the risk potentials of certain specific compounds. In addition, the program offices are charged with providing insights and guidance on the human health and environmental impacts of materials that enter the consumer market but do not necessarily conform to the existing testing guidelines (*e.g.* micro-particles associated with nanotechnology). Toward that end, one of the critical missions of NHEERL laboratories is to be responsive to the program offices' needs in dealing with emerging issues, such that timely findings from our targeted research projects can be used to provide a sound scientific basis (e.g., defensible science from peer-reviewed publications) to support OPPTS's risk assessment efforts. Because its mission is to advance the scientific basis informing EPA's decisions ORD does not conduct routine testing of agents but rather targets those with new or novel properties and for which study will likely yield new understandings about chemical risk that can be applied more broadly to other classes of compounds. In addition, OPPTS and ORD need to establish a formal process to identify and prioritize the emerging critical health and environmental issues that can be addressed by the ORD laboratories in a coordinated manner.

One of the critical missions of NHEERL laboratories is to be responsive to the program offices' needs in dealing with emerging issues by targeting those chemical classes with new or novel properties and for which study will likely yield new understandings about chemical risk that can be applied more broadly to other classes of compounds

Scientists from OPPTS have identified two major research issues that require immediate research support from NHEERL (and to a less extent, NERL) at ORD:

a) potential health risks of perfluoroalkyl acids (PFAA) in humans and wildlife; and
b) effects of high potency herbicides on non-target plants

Because of the disparate nature of these issues, each is addressed individually (*vide infra*). For each, specific problems facing OPPTS are described, and scientific questions are framed with brief outlines of the approaches proposed to address that question. Detailed research plans with specific aims, estimated FTE requirements and anticipated products for FY'05-FY'12 have been prepared and can be viewed on the NHEERL SP2 website:

http://www.nheerl.epa.gov/nheerl_science/pesticide_implementation/.

Additionally, a preliminary plan is proposed for identifying the emerging or newly discovered hazards that come to the Agency's attention, but the formal mechanism will require several iterative deliberations among investigators at NHEERL as well as collaborators in other ORD laboratories. The critical paths for LTG 4 efforts are captured throughout the text.

Perfluoroalkyl Acids (PFAA)

Perfluoroalkyl acids (PFAA) and their derivatives are organic fluorochemicals that possess unique surfactant properties and have wide industrial and household applications, including coatings for fabrics and paper products, fire-fighting foams, electronic etching baths and insecticides. This class of man-made compounds is composed of members with various carbon chain lengths (primarily from C-4 to C-14), in the form of carboxylic or sulfonic acid. Some of the PFAA widely used in consumer products and/or commonly found in the environment are listed as follows.

Perfluorobutane Sulfonate (C-4, PFBS)
Perfluorohexane Sulfonate (C-6, PFHS)
Perfluorooctane Sulfonate (C-8, PFOS)
Perfluorooctanoic Acid (C-8, PFOA)
Perfluorononanoic Acid (C-9, PFNA)
Perfluorodecanoic Acid (C-10, PFDA)
8:2 Telomer Alcohol (8:2 TA)
10:2 Telomer Alcohol (10:2 TA)

The most prominent representatives of these chemicals are the so-called "C-8 compounds", Perfluorooctane Sulfonate (PFOS), Perfluorooctanoic Acid (PFOA) and the 8:2 Telomer Alcohol. Functional derivatives of these chemicals such as alcohols, esters and amides are used commercially, but these intermediates ultimately will break down to PFOS and PFOA as end-stage metabolites and products. These perfluorinated chemicals are very stable in the environment and have generally been considered biologically *inactive*; thus, concerns for potential hazards of these chemicals to human and ecological health have been minimal. However, recent documentation of the extensive (global) distribution and persistence of PFOS and PFOA in both humans and wildlife (biological half-lives in humans being estimated between 4-9 years) has raised considerable concerns from the regulatory agencies and the public. Indeed, most recent bio-monitoring studies that indicated the presence of other perfluorochemicals (C-6, C-9) in humans and polar bears further lend support to the findings with the C-8 chemicals.

Mammalian (primarily rodent and monkey) studies with PFOS and PFOA have suggested that hepatotoxicity (hypertrophy, peroxisome proliferation), carcinogenicity (liver, Leydig cell, pancreatic and mammary gland tumors), immunotoxicity (PFOA), developmental toxicity (perinatal mortality, delays in growth and development) and reproductive toxicity (delays in sexual maturation) might be associated with exposure to these chemicals (Lau *et al*., 2004; Butenhoff *et al*, 2004; Kennedy *et al*., 2004). In addition, thyroid hormone imbalance has been reported with PFOS and perfluorodecanoic acid (PFDA) exposure; while potential alterations of other hormones may be involved in the ovarian hyperplasia, mammary gland adenoma and Leydig cell tumors seen with PFOA treatment.

A key characteristic of PFAA toxicity is a steep dose-response relationship. Although little is known about the modes of action (MOA) for PFAA and the mechanisms of their toxicity are largely undefined, the involvement of the peroxisome proliferator-activated receptor (PPAR) pathway has been implicated. Indeed, most if not all PFAA (such as PFOA, PFNA, PFDA) are potent peroxisome proliferators, and PPARα in particular has been shown to play a pivotal role in promoting hepatic tumors in rodents. On the other hand, the reduced expression and peroxisomal proliferative activity of PPARa in human liver cells were considered important components in the risk assessment for tumorigenicity of PFOA. Based on these findings, PFOA activation of PPARa was deemed not

likely to play a role in human liver tumors. Yet, the potential involvement of PPAR in other PFAA-induced adverse outcomes remains to be determined. For instance, studies with PPARa knock-out mice indicated that PPARa might play a major role in the immunotoxicity caused by PFOA. Extensive investigation on the PPAR family of transcriptional factors has revealed critical roles for PPARa, PPARß/δ and possibly PPARγ in reproduction and development (*vide infra*). Because the PPAR pathways are clearly important for normal growth and development of the fetus and neonate, it is reasonable to postulate that disruption of these intracellular signals by PFAA may alter developmental processes and thereby contribute to adverse health effects.

Because of concerns for its potential adverse health effects, PFOS production was phased out by its major manufacturer 3M at the end of 2002, although small quantities of the chemical are still available from the oversea markets. More importantly, because of the versatility of these fluorochemicals in commercial applications, other PFAA substitutes have emerged to replace PFOS. The telomer-based PFOA (manufactured by DuPont) has been widely used in consumer products, while the PFAA of various carbon chain lengths (C-4, C-6) have been marketed by 3M as PFOS replacements. Uses of other PFAA (such as C-9 and C-10) are known to be under development. Indeed, according to the most recent findings reported at an international symposium (FLUOROS, 2005), C-9 has been widely detected in the wildlife populations, the levels found in some fish were as high as those for C-8 chemicals.

Accordingly, EPA is interested in assessing the health risks of PFAA because of

(a) their global distribution and persistence in the environment;
(b) their presence and accumulation in humans and wildlife;
(c) their toxicity findings with laboratory animal models;
(d) a virtual lack of information on modes of action (MOA) with regard to their toxicity;
(e) the paucity of knowledge about their replacements currently in commerce or under development.

Prioritization

A critical path describing our overall research approaches with the perfluoroalkyl acids is illustrated in Figure 6. Research projects involving hazard characterization and MOA for toxic outcomes, as well as pharmacokinetic studies are of top priority in this proposed multi-year research plan because findings from these projects will be instrumental to the risk assessment of PFAA by OPPT. The projects to ascertain the physiological significance of thyroid hormone imbalance produced by PFAA will address an overarching issue of endocrine disruption. Hence, products from these projects will also lend support to GPRA Goal 8.3 (Endocrine Disruption) research efforts. Projects to determine the structure-activity relationship (SAR) and cumulative risks of PFAA are lower on the priority primarily because of a lack of expertise and support currently available to NHEERL laboratories. Remedy of this situation and together with the advances of PFAA research in the coming years, undertaking of these projects should be readily feasible.

PFAA are composed of a dozen or so of known chemicals. Clearly, it is not within the scope of the multi-year implementation plan to examine every one of them. Hence, evaluations of these chemicals are ranked as followed:

Chemical	*Priority*	*Rationale*
PFOS (C-8)	high	Present in humans and wildlife, ubiquitous, persistent
PFOA (C-8)	high	Present in humans and wildlife, substitute for PFOS
8:2 TA	high	Present in humans and wildlife, substitute for PFOS
PFHS (C-6)	medium	Present in humans
PFBS (C-4)	medium	Substitute for PFOS, faster clearance
PFNA (C-9)	medium	Present in wildlife, environmental contaminant
PFDA (C-10)	low	Potential Environmental contaminant
10:2 TA	low	Potential Environmental contaminant
Other PFAA	low	Little to no information is available

Problems, Products and Impact.

Because PFOS production by 3M was phased out under a Consent Agreement with EPA, a human health risk assessment for this chemical is not required immediately by OPPTS. However, uncertainties about the adverse outcomes from exposure to PFOS remain a major concern to the Agency, particularly in view of its abundance and persistence in the environment as well as its presence and accumulation in human and wildlife populations. Moreover, because of the unique physical and chemical properties of these perfluorinated compounds, existing models for toxicity estimates and health risk assessment (for instance, those designed for the persistent organic pollutants) are not applicable. Hence, new information regarding hazard identification for PFOS is desirable in order to bolster the Agency's IRIS database on this chemical. Furthermore, information about its modes of action and mechanisms of toxicity is needed to support the Program Office's regulatory actions in the future.

Uncertainties about the adverse outcomes from exposure to PFOS remain a major concern to the Agency, particularly in view of its abundance and persistence in the environment as well as its presence and accumulation in human and wildlife populations

Because PFOA is poised to fill the void left by PFOS in commerce, a risk assessment of the potential human health effects associated with exposure to PFOA was drafted by OPPT in 2004 and reviewed by the SAB (February, 2005). The SAB concluded that an adequate risk assessment of PFOA would require substantial new information to fill the data gaps. While a final report from the SAB is pending at the time of the writing of this Implementation Plan, recommendations for immediate research needs from their interim draft report include:

a) Evaluation of mechanisms of PFOA carcinogenesis, in addition to the involvement of PPARa pathway;
b) Evaluation of non-cancer endpoints, notably the immunotoxic, neurotoxic and endocrine disrupting effects of PFOA;
c) Full evaluation of PFOA on the PPAR family, in addition to PPARα;
d) Investigation on the mechanisms of PFOA toxicity;
e) Elaboration of PFOA developmental toxicity, including critical periods of vulnerability, and on potential enhanced vulnerability of the aged;
f) Better understanding of PFOA pharmacokinetics in animal models and humans;

g) Studies on cumulative toxicity of PFOA and related PFAA;
h) Bio-monitoring of human exposure, with special focus on developmental life stages; and
i) Extensive evaluation of health effects of PFOA in occupationally exposed populations

The NHEERL research plan is designed to address these recommendations for PFOA and to provide a comprehensive evaluation of the entire class of PFAA (focusing on those whose presence in the environment has been documented). This proposed strategy includes three phases:

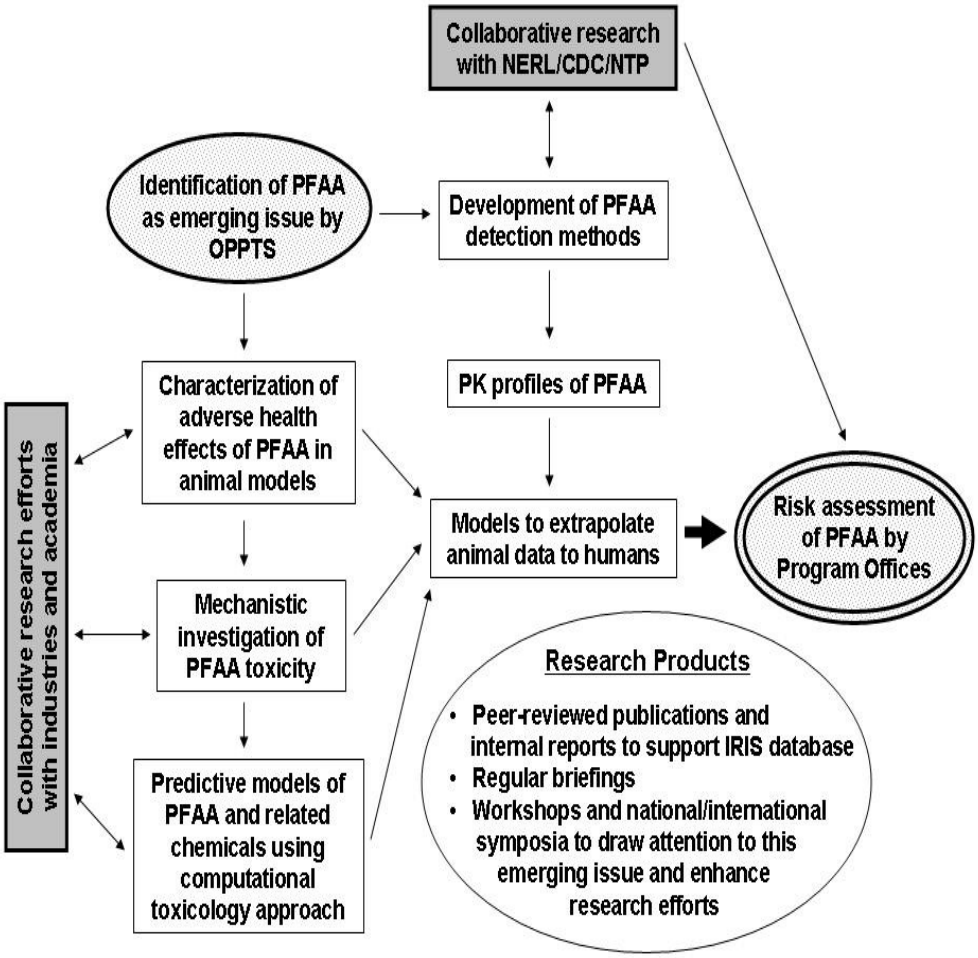

Figure 6. Critical path of NHEERL research under LTG4 Program Project Area 8: PFAA.

(i) short-range research (2-3 years) to address the immediate needs of OPPT regarding the risk assessment of PFOA, 8:2 TA and to a lesser extent, PFOS, by filling some of the data gaps identified by the Program Office and the SAB;
(ii) intermediate range research (3-5 years) to identify the MOA and the mechanisms of toxicity for some of the PFAA (for instance, PFOA, PFOS, PFHS, PFNA) in order to determine if there are common features shared by the entire class of chemicals; and
(iii) long-range research (5-8 years) to construct risk assessment models for this class of chemicals as well as others that may share similar chemical and physical properties.

These three phases are not designed to be stand-alone but will be cross-cutting and iterative. For instance, the short-term studies on hazard characterization of PFOA and PFOS will provide linkage to the MOA and lay the groundwork for mechanistic investigations that will require more sustained research commitment. As information and databases become available over time, our investigators will begin to construct models (such as comparative pharmacokinetics and structure-activity-toxicity relationships) to predict the potential adverse effects of perfluorochemicals that have not yet been marketed (for instance, the C11-C14 PFAA), and to address the more complicated issues of cumulative risks of PFAA. In addition, as the Program Office is moving to use the "Margin-of Exposure" (MOE) paradigm for risk assessment, accurate descriptions of PFAA body burden in our animal models will be essential for comparison to that in human estimates derived from bio-monitoring studies such as NHANES from CDC. Hence, some of our long-range efforts will be devoted to the development of analytical methods for detection of PFAA in various matrices in our animal studies, thereby providing body burden estimates as an internal dosimeter.

Critical elements of each phase of research are summarized below, with details provided in the body of this document.

Short-range Research: Hazard Characterization of PFAA.

a) Dose-response evaluation of the developmental toxicity of PFOS, PFOA, 8:2 Telomer Alcohol, and PFHS in appropriate animal models for extrapolation to humans: *these chemicals have been detected in humans and PFOS is a developmental toxicant in several animal species tested, expanded investigations of non-cancer endpoints will provide OPPT with bench mark dose (BMD) estimates for the IRIS database and risk assessment*;
b) Dose-response evaluation of the immunotoxic potentials of PFOA and PFOS: *our investigation will substantiate and clarify the confounding results from the limited studies currently available in the literature, and to provide BMD estimates for the IRIS;*
c) Evaluation of the mammary gland and Leydig cell tumors reportedly induced by PFOA: *our study will clarify these controversial findings that limited the risk assessment of PFOA;*
d) Examination of the thyroid hormone-disrupting effects of PFAA: *investigations will substantiate the novel findings of thyroid hormone changes in our preliminary studies and determine whether these effects would constitute a health risk; these research efforts will be coordinated with ORD's Endocrine Disruption Program.*
[*Pilot studies of some of these projects have already been initiated, while others are in final planning stages.*]

Intermediate-range Research: Modes of Action for PFAA Toxicities.

a) Elucidation of the pathophysiological mechanisms of the adverse developmental effects of PFOS and PFOA: *these studies will pin-point the targets and manners of chemical insults and facilitate the assessment of human health risk; in addition, identification of the mechanisms of toxicity for these C-8 chemicals will provide a template for the evaluation of other PFAA*;
b) Exploration of the roles of PPAR signaling pathway in the expression of PFOA toxic outcomes (developmental toxicity, immunotoxicity, hepatotoxicity): *our studies will determine if the PPAR pathway may provide a plausible mechanism for the multiple toxic manifestations of PFOA, this mechanism will be explored relative to other PFAA;*

c) Evaluation of the potential involvement of altered steriodogenesis (and the associated hormonal imbalance) in PFOA-induced mammary gland and Leydig cell tumors, and ovarian hyperplasia: *if the short-term investigations confirm tumor inductions, follow-up studies will be conducted to explore the underlying mechanisms of this adverse effect;*

d) Exploration of the underlying mechanisms responsible for the endocrine disrupting effects of PFAA: *if the thyrotoxic effects of PFAA are confirmed, additional research will be carried out to pin-point the etiology of hormonal imbalance and their implications to human health.*

Long-range Research: Construction of Risk Assessment Models for PFAA.

a) Development of analytical methods to assess the body burdens of PFAA in our animal studies: *accurate measurements of internal dose of PFAA in our animal studies will be crucial for an MOE estimate for human health risk assessment;*

b) Construction of comparative pharmacokinetic models with various animal species for extrapolation to humans: *these models will lend support to the extrapolation of data from animal studies to human health risk assessment;*

c) Exploration of gene expression approaches to identify the MOA for PFOA and PFOS toxicities, and potentials of these approaches in the construction of SAR models: *these studies will capitalize on cutting-edge genomics and molecular biology tools to explore the MOA of the C-8 chemicals and to provide databases for SAR models in order to predict the toxic potentials of other PFAA, as well as the aggregate/cumulative risk potentials of PFAA.*

The scope of these research plans is ambitious and requires collective efforts from a team of principal investigators with interdisciplinary expertise in reproductive and developmental toxicology (RTD), immunotoxicology (ETD), developmental neurotoxicology and thyroid function (NTD). We also anticipate extensive collaborations with other laboratories within ORD (such as NERL at RTP, NC and Athens, GA), and researchers from academic institutions, industries and other government agencies. For instance, our (RTD) joint efforts with investigators from the Human Exposure and Atmospheric Sciences Division of NERL and 3M scientists are instrumental for establishing analytical methods to detect PFOS and PFOA in serum and tissues from our animal studies. Furthermore, these efforts will be coordinated with other research institutes such as CDC. This will ensure cross-platform compatibility in order to facilitate extrapolation of data from animal studies to human exposure. In addition, our research plans with PFAA will be coordinated with efforts from the National Toxicology Program (NTP) to maximize our resources and to strengthen our strategies. Our research directions, progress and findings will be communicated to OPPT on a regular basis through internal reports, briefings and workshops, and guidance will be sought routinely to confirm and address the programmatic needs.

In general, our products will be comprised of peer-reviewed journal publications and internal reports submitted to OPPT. Results generated from our studies will be incorporated into the Agency's (as well as OECD's) IRIS database and provide a cornerstone to the risk assessment issued by the Program Offices. Specific products and time-line of delivery (APM) are described for each individual research approach (*vide infra*). The impacts of this research are two-fold:

a) to provide OPPTS with a sound and credible database for the human health risk assessment of the C-8 chemicals in the immediate future, and other PFAA thereafter; and

b) to provide a better understanding of the MOA for PFAA toxicities, thereby affording improved designs for safer substitute products for this important class of compounds.

High Potency Herbicides

Introduction:

The MYP LTG 4 research will provide protocols necessary to address the risks of novel or newly-discovered potential hazards to plants. The focus is on low-dose, high-potency herbicides, which are defined as those herbicides with a maximum label application rate of 0.5 pounds of active ingredient per acre (560g/Ha). Several classes of chemicals fall into this category, including the acetolactate synthase (ALSase) inhibitor herbicides, imidazolinones, sulfonylureas, triazolophyrimidine sulfonamides, isoxaflutoles, and pyrimidyl thio-benzoates. Chlorsulfuron, an ALSase inhibitor, was the first commercial product of this type, first marketed in 1981. There is evidence for a wide variation in response of various plant species to these chemicals (about 3 orders of magnitude), making it difficult to extrapolate potential risk from the current suite of plant test species. Given the increasing use of these types of herbicides, in 2001 the FIFRA Science Advisory Panel recommended restructuring the Tier II risk assessment process to expand the number of species tested and the endpoints evaluated. Thus, we will focus on improved Tier II testing protocols for determining phytotoxicological responses to low-dose, high-potency herbicides, and potentially to other novel hazards. However, recognizing the potential importance of indirect and direct effects of novel hazards on plant communities, we will go beyond Tier II tests to evaluate and develop Tier III protocols to determine ecological effects to low-dose, high-potency herbicides. We also will develop chemical/molecular testing protocols to provide *in vitro* methodologies to characterize risks effects of low-dose, high-potency herbicides to species beyond those initially tested and for herbicides with similar modes-of-activity.

The science will produce improved protocols for detecting reproductive, ecological, and chemical/molecular effects on plants, and spatial analysis tools to

Critical Path:

Figure 7 illustrates how the research identified in this plant addresses agency problems in three areas relating to risk assessments for novel herbicides in terms of reproductive responses, ecological responses, and chemical/molecular detection of effects. The science questions raised by these areas of interest are identified as well as the scientific approach proposed to address those questions. The research products are improved protocols for detecting reproductive, ecological, and chemical/molecular effects on plants, and spatial analysis tools to conduct risk assessments. Finally, the strategic products will be improved Tier II and III testing protocols for ecological risk assessments and chemical/molecular tests to assess the potential effects of novel herbicides in the field. and for different species.

Problems, Products and Impact:

Problems:

Pesticide drift to unintended fields is inevitable and the magnitude of potential and the effects to non-target plants are highly variable over time and space. OPP is mandated under FIFRA to evaluate the potential ecological risks of crop pesticide drift to non-target plants. Specific research is needed to assess the potential impact of pesticide drift and to understand the effects on individual plants and higher biological assemblages across the landscape.

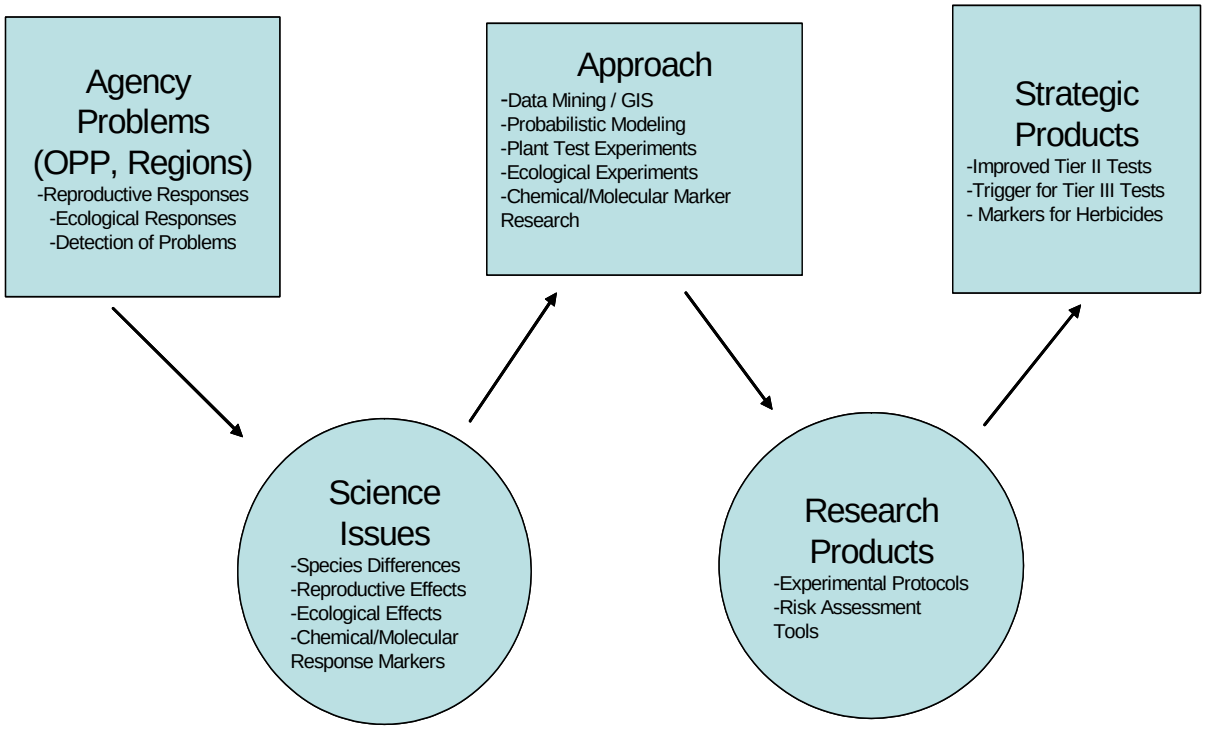

Figure 7 Critical Path for low-dose, high-potency herbicide research

The rationale underlying the proposed research on herbicide drift effects is that non-target crops and native plants in terrestrial or aquatic systems in close proximity to target crops are likely to be exposed, and to respond to, chemical pesticides. Herbicide drift generally occurs within 300 m of the crop field margin during and shortly after application (SDTF, 1997b). Drift amount and location depends upon the application rate and method (ground or aerial), environmental conditions, droplet spectrum, application height, and distance from the field boundary (Bird et al., 2002). Vegetation located at the immediate boundaries of agricultural crop fields is at greatest risk from drift (Teske et al., 2002). The magnitude of herbicide drift effects on plant productivity or community composition depends upon the amount and type of herbicide and plant sensitivity at time of exposure, which varies with plant species and its phenological stage. In addition to plants near target crops, aquatic vegetation could be affected by transport of herbicides in water downstream from the area initially affected. However, the effects of herbicide in water are beyond the scope of the current implementation plan.

In order to improve risk assessments for nontarget effects of novel herbicides on plants, it will be necessary to characterize those risks in a probabilistic framework, i.e., by presenting a range of possible effects based on a range of characteristics of exposure and of the target organisms, and not just the deterministic results based on simple herbicide exposure / plant effects functions. This means that assessments would give a range of outcomes given a range of exposure-effects profiles. The existing databases represent potentially

In order to improve risk assessments for nontarget effects of novel herbicides on plants, it will be necessary to characterize those risks in a probabilistic framework

large sources of variation because the data were not specifically designed for regional risk assessment and, consequently, the data supports had low spatial and/or temporal resolution. The most important data gaps in terms of potential effects are lack of tests for reproductive responses of plants, lack of information of ecological responses, and lack of methodologies to extrapolate results among a wide range of potentially impacted plant species. Research needs to be conducted to develop new methodologies related to improving the effects component of the assessment process in terms of phytotoxicity tests, ecological tests, and chemical/molecular tests, respectively.

Products/Impact:

We will improve models and databases to determine ecological risks of pesticide use (especially on low-dose herbicides) to other plants in a spatially explicit landscape. We will produce comprehensive and efficient *in vivo* assays to evaluate adverse effects of chemical herbicides at critical plant life stages, and will develop new approaches for tier II testing, including methods for native plant species. We will develop guidelines for Tier III which may be required on a case-by-case basis. We will develop protocols for chemical/molecular tests to characterize risks to species beyond those initially tested and for herbicides with similar modes-of-activity. The focus of our research will be on specific products which will impact terrestrial plant risk assessments, with limited research on products for aquatic plant risk assessment. However, much of the new knowledge gained regarding terrestrial plants could be applied to aquatic plant research in the future.

Science Questions

Species Selection. In terms of species selection for basic Tier I and II phytotoxicity tests, the narrow taxonomic and life form range of the ten currently required test species for terrestrial plants and only *Lemna spp.* for aquatic plants, raises the question of whether the majority of plant species will be protected, including all the rest of the angiosperms (both herbaceous and woody), gymnosperms, and ferns. For terrestrial plants, six dicotyledonous from four families including soybean and a root crop and four monocotyledonous from at least two families including corn. All other species are only recommended, and substitution of other test species (other crops, weeds, native plants, perennials, woody species) especially are encouraged only when species sensitivity to the test compound is known ahead of time. In practice, all ten species usually are annual agricultural species. Maize and soybean are required, and a root crop (often carrot), plus tomato, cucumber, lettuce, cabbage, oat, ryegrass and onion usually are used. In terms of aquatic plants, *Lemna* is a small, floating, freshwater, monocotolydenous plant which may not represent the susceptibility of the wide range of aquatic species found in different types of aquatic ecosystems (e.g., submerged, emergent, floating).

In reality, plant species vary greatly by ecological regions and taxonomy, yet there is no provision for including this diversity in non-target plant risk assessments. Of particular concern for terrestrial plants is the possibility that native or nonagricultural species may be more sensitive to herbicides than the crop species usually used in the Tier I and II tests. A recent analysis of relative susceptibility of species used in herbicide testing McKelvey et al. (2002), countered this suggestion and reported that the crop species currently used in vegetative vigor tests usually were more sensitive than non-crop plants tested. However, McKelvey et al. (2002) only considered "weed" species (primarily annuals) as non-target plants and only used high (several x the field application rate) herbicide concentrations. Furthermore, even though the potential effects of a pesticide on

threatened and endangered plant species is one of the primary concerns for risk assessments, there is procedure for evaluating these effects using surrogate species or other methodologies. Similar concerns exist for aquatic plant species which may different exposure characteristics in terms of different life forms in addition to different responses based on species.

In terms of Tier III ecological tests, there are general guidelines for the types of terrestrial or aquatic plant species to be used, but no systematic approach for selection of those species to meet specific risk assessment needs.

Phytotoxicity Tests. While the existing Tier I and II test protocols used to determine non-target terrestrial plant effects were established using the best available scientific information at the time, they do not reflect subsequent methodological questions or advances in scientific methodology.

The Tier II vegetative vigor test lasts a maximum of 28 days. This time period is insufficient to capture the reproductive phase of the plant's life cycle. Early plant growth effects may not predict latent adverse reproductive effects as limited available data suggests that herbicide exposure during the vegetative versus reproductive phases may not have equal influences on reproduction and crop yield (Fletcher et al., 1996). Not only is this important for the individual plant's ability to pass along its traits, but reproductive yield is one of the most important economic aspects of agriculture. Furthermore, many wildlife species depend upon seed production of noncrop plants for their food source.

Current Tier II tests include only measurements of injury, height and biomass that do not correlate well with reproductive responses (e.g., crop yield). These short-term, vegetative growth endpoints (biomass, height), while useful to indicate general lethality of herbicides, may not provide data regarding longer-term effects on plants such as sublethal effects, the ability of plants to recover from stress, or changes in the competitiveness of plants. It can be argued that early seedling growth parameters are protective of reduction in yield responses, but this may lead to over regulation of some herbicides. Maxwell and Weed (2001) and Obrigawitch et al. (1998) point out potential limitations of current and possible alternative assessment indicators for nontarget effects of herbicides. Obrigawitch et al. (1998) specifically assessed nontarget effects of sulfonylurea herbicides using field approaches. They concluded that while the risks to non-target plants from sulfonylureas were similar to those from other herbicides used at higher application rates, there was a the need for standardized protocols to assess the effects of herbicides, in general, on non-target plants.

Most significantly in terms of novel newer herbicides, current plant testing protocols initially were developed based on information regarding effects of wider-use but lower-potency herbicides such as 2,4-D. In contrast, much of the recent interest in herbicide testing has been associated with low-dose, high-potency herbicides such as acetolatacte synthase (ALSase) inhibitor herbicides which where initially introduced into U.S. agriculture in the mid-to-late 80s (Fairbrother and Kapustka, 2001). Use of the newer chemicals potentially addresses major environmental concerns regarding herbicide toxicity in that they have a relatively narrow spectrum of susceptible organisms, are relatively short-lived in the environment, nonbioaccumulative and used in low volume. The first class of these herbicides used was the sulfonylureas (SU). They were quickly followed by the imidazolinones and more recently by triazolopyrimidine sulfonanilides and pyrimidinyl oxybenzoates. The primary mode of action of these herbicides is by disruption of the synthesis of the branched chain amino acids leucine, isoleucine and valine. However, there may be secondary modes of action within plants leading to the accumulation of toxic metabolites, disruption of

assimilate transport and inhibition of reproduction (Fairbrother and Kapustka, 2001; Taylor, 2001). These herbicides are generally not considered to be toxic to animal systems due to animals' inability to synthesize branched chain amino acids. These ALS inhibitors can affect bacteria and fungi which play key ecological roles in nutrient cycling, soil fertility, and plant nutrition and health. From past experience with other pesticides, from information presented at a recent workshop on low-dosage-herbicides in December, 1999 (Ferenc, 2001), and based on findings from the FIFRA Scientific Advisory Panel Meeting in June, 2001, there is general agreement that the low-dose herbicides are moving off site in water, on soil particles and as spray drift. For example, the use of Oust® (sulfometuron) on rangeland may have resulted in damage to potatoes at least 8 km distant (personal communication, Dr. Pamela Hutchinson, University of Idaho, Aberdeen Research and Extension Center).

There are a number of reasons why low-dose, high-potency herbicides such as the ALSase inhibitors may be of greater interest than conventional herbicides. As summarized by Maxwell and Weed (2001), compared with conventional herbicides, low-dose, high-potency herbicides may: 1) have increase in amount if current use trends continue, 2) have greater aerial drift because of their application methods, 3) be used more at the boundaries of agricultural regions as they become used more for roadside maintenance, 4) be used more to suppress forest understory plants in forest ecosystems, and 5) have more potential for reproductive effects due to exposure amounts and timing for different crops. In his preliminary ecological risk assessment and characterization for ALSase inhibitors, Taylor (2001) concluded that "…the uncertainty of the data is significant in terms of breadth and depth…"

Thus, research is needed to develop new phytotoxicity tests to develop endpoints most important for the herbicide risk assessment process. These tests must address the three key research needs: i.e., the relationship between the time of exposure during a plant's life cycle, reproductive and development endpoints of particular usefulness when evaluating responses crops and native plants, and how different classes of chemicals affect various specific reproductive or developmental responses. The tests must also be applicable to a wider array of plant families and species identified at risk through spatial analysis. Finally, besides their inherent importance in indicating phytotoxic effects to individual plants, the Tier II tests must be as accurate as possible as significant Tier II effects are an important trigger for the requirement for broader ecological tests.

Research is needed to develop new phytotoxicity tests to develop endpoints most important for the herbicide risk assessment process

Ecological Tests. Even though movement of herbicides from targeted land has the potential to adversely affect not only individual plants, but both agricultural and natural plant communities, there has been little development of protocols for ecological effects (Tier III) tests. The ecological effects may be direct, such as the elimination or reduced reproductive output of certain plant species in a community which leads to the alteration of the community's species composition, structure and function. Effects may also be indirect, such as changes in microbial communities, controlling plant pathogens, or diminished insect populations causing wildlife populations to decrease. Both the direct and indirect effects could lead to numerous negative impacts on wildlife habitat, nutrient cycling, control of soil erosion, recreation, timber or pulp production, livestock grazing, control of noxious plant species, and aesthetics (Obrigawitch et al., 1998). As early as in 1991, the US SAP

recommended that: "...community response measures need to be developed that have the potential to identify significant structure and functional changes in exposed communities. Many more invaded natural communities will be targeted for herbicide use, given the increased recognition of invasive plant problems. Community response metrics are available in the ecological literature. However, the specific value of these responses with regard to characterizing responses due to chemical exposures need to be determined and possible modifications of designs identified." However, tests have not been adequately developed to determine such ecological responses.

In terms of direct effects, data suggest that plant assemblages at field margins experienced substantial change in species frequency and distribution due to differential susceptibility to herbicides (Kleijn and Snoeijing, 1997). Others (Jobin et al., 1997) have found lower species diversity in the herbaceous layers of hedgerows and woodland edges of cultivated fields with a history of herbicide use as compared with those near fields without herbicide use. In controlled experiments with plant communities, Pfleeger and Zobel (1995) demonstrated that variable species responses to herbicide exposure alter the competitive interactions within a community.

The high selectivity of the low dose, high potency herbicides could accentuate the differential stresses and subsequent shifts in dominance in a plant community. Such shifts in a community can result in changes in frequency and production and even extinction of desired species (Tillman, 1988). Boutin and Jobin (1998) demonstrated that herbicides can contribute to shifts in plant communities adjacent to intensively cropped fields from native species toward more weedy species, and, thus, these adjacent communities can promote the spread of weed species. Additionally, crops are being genetically engineered to be tolerant to the highly active herbicides, which will stimulate more widespread use and subsequent potential for non-target effects (Maxwell and Weed, 2001).

Determination of risks to threatened and endangered plant species in the native communities is critical. In the US, the federal government has listed over 500 plant species and the Nature Conservancy considers 5000 of the 16,000 native species to be at risk. Almost 50% of these species are annuals that are dependant on seed production or the seed bank for survival. The highest percentage of these plants is located in Southeast wetlands and Southwest deserts.

Determination of risks to threatened and endangered plant species in the native communities is critical

Indirect effects of herbicide drift to plants can affect the complexity of food web dynamics, and, thus, wildlife populations. The vast majority of the reproductive output of plants is used by animal species as sources of energy. Therefore, changes in plant community dynamics will affect wildlife populations. Freemark and Boutin (1995) indicated that there is evidence that effects of herbicides on plants can affect wildlife. For example, populations of the gray partridge in the United Kingdom have been affected by herbicide use (Greig-Smith, 1991). Plant species composition within hedgerows between herbicide sprayed fields was altered, resulting in a 50% loss in populations of arthropods, which were a high-protein food source for partridge chicks. Fewer arthropods resulted in more frequent partridge brood movements leading to greater predation of chicks.

Freemark and Boutin (1994) summarized the impacts of herbicides on biotic communities by stating: "*Different taxonomic groups…play important roles in agroecoystems in soil fertilization and aeration, the recycling of organic material and nutrients and the degradation of contaminants.…The use of agricultural herbicides (and other pesticides) can interfere with these functions by altering plant biochemistry, developmental processes and morphology,*

changing population dynamics, species composition and diversity, interrupting energy and nutrient flows, degrading water quality and changing the composition, heterogeneity and interspersion of habitats for wildlife."

Thus, because the data available on ecological effects of herbicides are very limited and highly speculative, there is a need for new designs for ecological effects (Tier III) tests. New tests for both direct and indirect effects to nontarget plant species are needed which build upon the herbicide phytotoxicity efficacy by including analyses of impacts on additional species of ecological interest. Considerable work will be required to identify an effective methodology for determining meaningful endpoints. For example, in evaluating the effectiveness of clopyralid in controlling the invasive non-native plant yellow starthistle (*Centaurea solstitialis*), Morghan et al. (2003) also measured impacts on mean plant cover and frequency for a variety of plant families found in a native bunchgrass and vernal pool community in the Central Valley of California. Among other native plant responses, they found that a native *Viola* species was negatively effected, but that the effect did not persist beyond the first year after herbicide treatment. Similar research should be conducted with efficacy tests for herbicides designed to control other invasive plant species in rangelands and other areas.

Ecological test protocols also are required to meet the modeling needs for probabilistic risk assessments. The tests would be field-based and include the measures of plant productivity and community structure that are vital for defining quality of wildlife habitat and the persistence of plant communities. An important emphasis of any ecosystem response protocol would be the provision for links to wildlife population models The ecological tests could be conducted at different scales depending on the scientific question being asked and available resources, and could range in scale from the simple to complex as follows:

Chemical/Molecular Tests. As currently conducted, the pre-registration evaluation of chemical herbicides or other chemicals considers a very narrow range in the genetic, economic and ecological breadth of organisms present in the highly diverse ecosystems found in the United States. We need to develop methods that can serve as biomarkers of ecological effects of environmental stresses (McCarty et al., 2002; McCarthy, 1990). On a whole-plant level, specific susceptible plant species have served as indicators or "sentinels" for herbicide drift due to their characteristic responses to low levels of herbicides (Felsot et al, 1996, Al-Khatib et al., 1993). However, future development of biomarkers should make use of new technologies in chemical analysis as well as molecular biology. They need to be specific for herbicide effects in plants, as such as cholinesterase inhibition as a biomarker of insecticide exposure in animals (Chambers et al., 2002).

Recent technical advances in plant genomics (e.g., Glombitza, S., et al. Plant Mol. Biol. 54: 817 - 835) especially support the concept of using microarray and other molecular diagnostic methods as additional tools in assessment and diagnosis of herbicide exposure of plants. Analysis of microarray gene expression data and subsequent compilation of plant responses to specific classes of herbicides will assist efforts to identify biomarkers for (a) plant exposure to herbicides and (b) identification of low dose, high potency herbicides such as the sulfonylureas, which currently cannot be readily identified by standard chemical methods. Information generated in these studies also is expected to lead to the development of biomarkers that are specific for plant reproductive and vegetative development effects resulting from herbicide exposure. The proposed research is expected to result in the development of very

sensitive and early biomarkers of plant exposure to these and other types f herbicides.

Such molecular biomarkers also would enhance the ability of EPA to better understand the effects of herbicides and other chemicals on target and non-target plant species at the molecular level.

LTG 4 Program Project Areas

Three Program Project areas have been identified under LTG 4 in this plan. The two major research issues are designed to provide immediate research support from NHEERL (and to a less extent, NERL) at ORD:

a) potential health risks of perfluoroalkyl acids (PFAA) in humans and wildlife; and

b) effects of high potency herbicides on non-target plants.

A third Program Project area has been identified entitled "Dealing with the emerging risks of new or existing pesticides and other chemicals." This project area addresses the need to establish a formal process to identify and prioritize new 'emerging risks'. This project will develop criteria to determine when to start new research and when to phase out or realign existing research efforts.

The research that will be conducted under LTG 4 is summarized in Table 6 and the project descriptions that follow. More detailed descriptions of these projects are found in Appendix C on the NHEERL SP2 website:

http://www.nheerl.epa.gov/nheerl_science/pesticide_implementation/

Table 6. Summary of NHEERL LTG 4 Program Projects			
Program Project Area 8: PFAA			
Approach	Title	Partners	Contact/Lead
A	*Characterize the developmental toxicity of PFOS, PFOA, telomer products and related compounds, and elucidate their modes of action*	NHEERL – RTD NERL – HEASD	C. Lau (RTD) J. Rogers (RTD)
B	*Development of risk assessment models: pharmacokinetic considerations*	NHEERL–RTD NERL–HEASD NCCT, NTP	C. Lau (RTD)
C	*Evaluation of immunotoxicity of PFAA responses*	NEERL – ETD	R. Luebke (ETD)
D	*Characterize the pathophysiological mechanisms of neonatal effects associated with PFOS and PFOA exposure.*	NHEERL – RTD 3M Univ. of Minn	J. Rogers (RTD)
E	*Elucidation of the Roles and significance of PPAR in developmental responses to PFAA*	NHEERL – RTD NHEERL – ETD	B. Abbott (RTD)
F	*Mechanistic investigation of tumor induction by PFAA.*	NHEERL – RTD	S. Fenton, G. Klinefelter,(RTD)
G	*Evaluate thyroid-disrupting potentials of PFAA and determine the significance of PFAA- induced thyroid hormone imbalance*	NHEERL – RTD NHEERL – NTD 3M	C. Lau (RTD) M. Gilbert (NTD)
H	*Development of risk assessment models: pharmacokinetic considerations.*	NHEERL – RTD NTP	C. Lau (RTD)
Program Project Area 9: **Effects of Novel Herbicides**			
A. Effects of Novel Herbicides	Project 1. Effects of Novel Herbicides-Spatial Analysis	NHEERL - WED	D. Olszyk
	Project 2. Effects of Novel Herbicides-Phytotoxicity Tests	NHEERL - WED	D. Olszyk
	Project 3. Effects of Novel Herbicides-Ecological Tests	NHEERL - WED	D. Olszyk
	Project 4. Effects of Novel Herbicides-Chemical/Molecular Tests	NHEERL - WED	D. Olszyk
Program Project Area 10: **Dealing with the emerging risks of new or existing pesticides and other chemicals.**			
	Project 1: Determining the priority for research with newly discovered hazards related to pesticides and chemicals		

Program Project Area 8: PFAA

Approach A: Characterize developmental toxicity of PFOS, PFOA, telomer alcohol products and related compounds, and elucidate their modes of action.

1. What is/are the key OPPTS problem(s) that this research effort will address?

Studies show that PFAA developmental toxicity may be a key feature for this class of compounds. More subtle effects on maturation of target organ systems and the effects of lactation exposure (i.e. high levels of PFOS detected in children) remain to be elucidated. PFOA developmental toxicity is seen in rats (U.S. EPA, 2002), but, unlike humans and primates, female rats clear PFOA efficiently (Kudo and Kawashima, 2003) rendering interpretation of the reproductive and developmental toxicity findings difficult. Thus, an alternative animal model with pharmacokinetic properties analogous to humans is needed for species extrapolation. Little is known about whether the 8:2 telomer alcohol itself may produce additional toxic effects beyond those associated with PFOA. Similarly, while other PFAA such as C-6 and C-9 are detected in humans and wildlife species respectively, virtually no information is available concerning their developmental effects.

2. What is the proposed research approach?

A follow-up study of the *in utero* exposure of PFOS will be conducted in an attempt to develop an alternative animal model (such as the mouse) lacking gender differences in PFOA clearance (similar to humans) and to re-evaluate the developmental toxicities of the chemical. The findings will be correlated with PFOA body burden for extrapolation to humans. Additional endpoints (e.g., mammary gland development, maturation of reproductive organs and functions) to bolster the evaluation of PFOS and PFOA developmental toxicity will be incorporated in our studies. Investigation will be extended to include 8:2 telomer alcohol, C-6, C-9 and C-10.

3. How does this research support the conceptual model for addressing this LTG?

It addresses OPPT's immediate need to characterize the hazards of these chemicals for human and wildlife populations. It is part of our short-range response to support to OPPT. Descriptive findings will help form the basis for the intermediate range research projects that focus on modes of actions for PFAA toxicity.

4. If this project is successful, what products or tools will result from the effort?

Primarily peer-reviewed publications to inform OPPT risk assessments. An accurate measure of the body burden associated with adverse outcomes in our animal models will allow OPPT to use the Margin-of-Exposure (MOE) paradigm for human health risk assessment of these chemicals. Specific information from our dose-response evaluations will be used for Benchmark Dose estimates in the IRIS database.

APM. Characterization of PFOA developmental toxicity in the mouse. (RTD/Lau)	**FY07**
APM. Determine the PFAA doses required for effects on the developing reproductive systems of male and female rodent offspring. (RTD/Fenton, Klinefelter).	**FY09**
APM. Evaluation of developmental toxicity of other PFAA. (RTD/Lau)	**FY11**

Approach B: Development of risk assessment models: pharmacokinetic considerations.

1. What is/are the key OPPTS problem(s) that this research effort will address?

OPPT needs pharmacokinetic models for PFOS and PFOA to provide a better understanding of the endogenous distribution and clearance of this class of chemicals and their relationship to adverse outcomes. Pharmacokinetic parameters are needed to help extrapolate findings from animal models to humans for the risk assessment of PFAA (for instance, estimation of margin of exposure based on body burden of PFAA).

2. What is the proposed research approach?

Collaborative studies will be set up with investigators at NERL to optimize methods to analyze PFAA with serum and tissues obtained from our rodent studies with PFOS, PFOA, and 8:2 TA. Our procedures will be shared with other leading laboratories (in academia, government and industry) so that results can facilitate animal-to-human extrapolation. As reliable analytical methods are established, we will develop pharmacokinetic and tissue dosimetry data and models to support interspecies extrapolation. RTD investigators will carry out the rodent pharmacokinetic and toxicity studies and NCCT investigator will develop the models. Studies will be carried out in mice and rats due to the dramatic species and sex differences observed in PFOA pharmacokinetics. If needed, similar pharmacokinetic studies can be extended to other PFAA such as C-6, C-9 and C-10 compounds.

3. How does this research support the conceptual model for addressing this LTG?

These studies will help provide the basis for a "Margin-of-Exposure (MOE) risk assessment through more accurate assessment of the exposure in humans and animals.

4. If this project is successful, what products or tools will result from the effort?

This project will help OPPT to understand the distribution of PFAA in the body and tissue level exposures in both experimental animals and humans providing the basis for more accurate exposure inputs and dose-response information for risk assessments.

APM. Pharmacokinetic profile of PFOA in the mouse. (RTD/Lau)	**FY08**
APM. Pharmacokinetic models for selected PFAA for interspecies extrapolation. (RTD/Lau)	**FY10**

Approach C: Evaluation of immunotoxicity of PFAA.

1. What is/are the key OPPTS problem(s) that this research effort will address?

The PFOA-induced immunotoxicity described in the OPPT risk assessment is based on findings obtained from one laboratory (Yang et al. 2000, 2001, 2002a,b) who reported reduced spleen and thymus size in mice exposed to PFOA as well as a >50% suppression of the IgM antibody response and 90% reduction of the IgG response. However, the paradigm used by Yang et al. is not a generally accepted method and some of their data suggests significant procedural inconsistencies. Further, these adverse immune system effects have not been independently corroborated. Lack of corroboration and the apparent mixed involvement of PPAR thus led the SAB to highlight the need for research to clarify these uncertainties.

2. What is the proposed research approach?

Yang et al.'s studies will be repeated with standard immunotoxic endpoints, and they will be extended to male rat for species comparison. IgM and IgG antibody responses will be evaluated in groups of animals exposed to PFOA before and after primary immunization. In addition, the effects of PFOA exposure on cell mediated immunity, expressed as the T-lymphocyte-driven delayed type of hypersensitivity (DTH) response, will be examined in separate groups of animals. If warranted, the same approach will be taken to extend the investigations to other PFAA. Furthermore, the immunotoxic potentials of PFOA will be evaluated in developing animals to determine if they represent a sensitive sub-population for the adverse effects of PFAA.

3. How does this research support the conceptual model for addressing this LTG?

This work represents a short to intermediate approach to identify the hazard potentials of PFAA (immunotoxicity) and to provide a better understanding of the cellular/molecular mechanism.

4. If this project is successful, what products or tools will result from the effort?

Findings from our studies will be published to enable OPPT to use our results in their risk assessment of PFOA and related chemicals. Our work will be critical in clarifying the current understanding of the adverse outcomes of PFOA and in determining whether these endpoints are appropriate for human health risk assessment.

APM. Determine the effects of PFOA exposure on the adult immune system. (ETD/Luebke)	**FY08**
APM. Determine the relative sensitivity of the developing immune system to PFAA. (ETD/Luebke)	**FY09**
APM. Determine the role of PPAR signaling pathway in PFOA-induced immunotoxicity. (ETD/Luebke)	**FY10**

Approach D. Characterize the Pathophysiological mechanisms of neonatal mortality and growth retardation associated with PFOS and PFOA exposure.

1. What is/are the key OPPTS problem(s) that this research effort will address?

The modes of action for PFAA adverse effects are virtually unknown, casting uncertainties in their risk evaluation. Early indications are that two salient features of PFOS and PFOA developmental toxicity in rodents include neonatal mortality and growth retardation. The underlying mechanisms and target organs responsible for these adverse outcomes remain to be identified.

2. What is the proposed research approach?

We will attempt to determine the critical window of susceptibility to PFOS-induced neonatal mortality, and assess the effects of PFOS on prenatal lung development. Lungs will be collected during the perinatal period and assessed for their degree of maturity by histological, morphometric, biochemical and molecular techniques. We will also measure surfactant proteins in lung lavage fluid from neonates to assess release of surfactant into the terminal air sacs. The involvement of altered hepatic functions such as lipid and cholesterol metabolism (possibly through the PPAR signaling pathway) in the PFOS- and PFOA-induced neonatal mortality and growth deficits will also be investigated and tightly coordinated with the research conducted under Approach F. Finally, gene expression analysis of a variety of markers of lung maturation, will be examined for clues into PFOS' MOA for lung and liver toxicity.

3. How does this research support the conceptual model for addressing this LTG?

The work described here will better define target organs and modes of action for the developmental toxicity of PFAA. This represents our intermediate range research that will rely on an initial description of the PFAA-induced adverse outcomes (Approach A) and interactions with other investigations (Approach E, for instance).

4. If this project is successful, what products or tools will result from the effort?

The products of this effort will be peer-reviewed journal articles identifying the MOA of PFAA developmental toxicity. These findings will allow OPPT to draw paralleled comparisons, based on MOA information, in their risk assessment of other perfluorinated alkyl chemicals in the future.

APM. Report on the critical developmental period for PFOS-induced neonatal mortality in the rat and the effects of PFOS on perinatal development of the lung in the rat. (RTD/Rogers)	**FY08**
APM. Identification of the MOA for PFOS and PFOA developmental toxicity by genomic and proteomic approaches. (RTD/Rosen)	**FY10**

Approach E: Elucidation of the roles and significance of PPAR transcriptional signaling in developmental responses to PFAA.

1. What is/are the key OPPTS problem(s) that this research effort will address?

The potential involvement of the peroxisome proliferator-activated receptor (PPAR), signaling pathway in the PFOA-induced liver tumor in animal model (and their significance in human health risk) is a major uncertainty PFAA risk assessment. Several PFAA (e.g., PFOA, PFNA and PFDA) are known to be potent PPAR agonists. Various PPAR isoforms (α, β/δ, and γ) have been implicated to play important roles in embryonic and fetal development, as well as placental functions. Do alterations of the PPAR signaling play a central PFAA toxicity MOA?

2. What is the proposed research approach?

We will construct transfected cell lines with a reporter gene to screen for the specific PPAR activities and expose them to various concentrations of PFAA to evaluate and compare their potency toward PPAR. Those PFAA with a high PPAR affinity will be investigated for their impact on growth by altering lipid homeostasis, fatty acid transport and metabolism, placental function, and hematopoiesis. Gene expression of the PPAR isoforms, co-factors and co-activators will be investigated in various embryonic, fetal and placental tissues, and patterns of changes in gene expression will be correlated with cellular endpoints indicating alterations in growth. Positive results will guide the third phase of our approach which will utilize a PPAR gene knock-out mouse model to confirm the PPAR-mediated effects.

3. How does this research support the conceptual model for addressing this LTG?

This effort represents our intermediate and long range research approach for this LTG. Results will provide information regarding the affinity of PFAA compounds for PPAR receptors and comparisons of the affinities of the compounds in rodent *vs* human receptors. This has the potential to provide valuable insights into the MOA for developmental toxicity, neonatal growth and survival. In addition, our findings will support the investigations of the pathophysiological mechanisms of PFOS and PFOA-induced neonatal mortality and growth deficits (Approach D) and the potential involvement of the PPAR signaling pathway in immunotoxicity (Approach C).

4. If this project is successful, what products or tools will result from the effort?

Our findings will be described in peer-reviewed publications and information from our dose-response evaluations can be applied to Benchmark Dose modeling for the IRIS database. This information will provide an improved understanding of MOA of PFAA that supports the risk assessment process and allows further evaluation of their relevance to human health. In addition, comparative potency of various PFAA on the PPAR activities can be used for future SAR and pharmacodynamic modeling described in Approach I.

APM. Effects of PFAA on developmental expression and activation of PPAR signaling pathways. (RTD/Abbott)	**FY09**
APM. Determine effects of PFAA on PPAR signaling functions that impact prenatal and postnatal growth regulation. (RTD/Abbott)	**FY11**

Approach F: Mechanistic investigation of tumor induction by PFAA.

1. What is/are the key OPPTS problem(s) that this research effort will address?

The carcinogenic potential of PFOA in the OPPT risk assessment is based on dietary exposure of the chemical in adult rats that indicated liver, Leydig cell, pancreas, and mammary tumors, in addition to hyperplastic growth in the prostate and ovary (Sibinski, 1987; Cook *et al*., 1994; Biegel *et al*., 2001). Currently, there are some controversies regarding the interpretation of these findings and a lack of mechanistic understanding of PFOA-induced tumor requires OPPT to use a linear default dose-response assumption that may over- or under-estimate the actual human health risk of this compound. Further investigation of these tumor incidence was called for by the SAB in their interim report on the PFOA risk assessment. In addition, it is unclear whether these tumors are unique to PFOA exposure or if they may represent broadly an adverse outcome to the exposure of the PFAA, as a class of chemicals.

2. What is the proposed research approach?

To clarify and confirm some of the tumor data derived from rat studies that have hampered the risk assessment of PFOA, liver, testis, ovary and mammary glands will be examined in mice exposed to the chemical in utero and through lactation. This research effort will be coordinated with NTP's bioassay after chronic exposure to PFAA (including PFOA). Once tumor incidence in these tissues are confirmed, we will shift our focus to determine if altered steroidogenesis might be involved in the tumor induction. P450 enzyme activity in these tissues will be monitored, along with circulating steroid hormone levels. The PPARα and non-PPARα components of liver tumor will be fully investigated with knock-out mouse model, in concert with research progress made in Approach E. A similar approach can be extended to examine the carcinogenic potentials of other PFAA.

3. How does this research support the conceptual model for addressing this LTG?

This short to intermediate range research will address the programmatic needs of hazard identification by assessing the carcinogenic potentials of PFOA (focusing only on liver, testis, ovary and mammary gland), and the potential involvement of a non-PPARα component in liver hypertrophy/hyperplasia. The latter issue will shed new light on human health risk assessment regarding liver tumor induction. Investigation of the PFAA-induced alterations of steroidogenesis not only will provide a better understanding of the MOA for these chemicals, but also may offer an alternative means in cancer risk assessment supported by mechanistic information such as endocrine disruption.

4. If this project is successful, what products or tools will result from the effort?

Our research findings will be communicated through publications in peer-reviewed journals to enable OPPT to use such information in conducting their risk assessment of PFOA as well as other PFAA in the future.

APM. Carcinogenic potentials of PFOA in mouse, possible involvement of endocrine imbalance. (RTD/Fenton)	**FY08**
APM. Report on MOA for tissue dysplasia/tumor formation following early life exposure to PFAA. (RTD/Fenton)	**FY11**

Approach G: Evaluation of thyroid-disrupting potentials of PFAA and determination of the biochemical and physiological significance of PFAA-induced thyroid hormone imbalance

1. What is/are the key OPPTS problem(s) that this research effort will address?

The unique thyroid-disrupting profile caused by PFOS raises two issues that OPPT must address: (a) whether the changes in circulating T4 and T3 (without corresponding feedback regulation of TSH) truly reflect an altered thyroid status, and therefore should be incorporated as an endpoint for risk assessment; and (b) if the thyroid-altering effects of PFOS might extend to other PFAA (such as PFOA)?

2. What is the proposed research approach?

To determine whether the thyroid-disrupting potentials of PFNA in the rat (Gutshall *et al*., 1989) and PFOS in the rat and mouse (Thibodeaux *et al*., 2003) represent a common feature for other PFAA, hormonal examination will be extended to PFOA, 8:2 Telomer Alcohol, and other PFAA of different carbon-chain lengths (C-4, C-6, C-9). These PFAAs were chosen based on their known presence in either human or wildlife populations. The mechanisms of thyroid hormone reduction in circulation will be elucidated by examining the effects of PFOS and related chemicals on the hepatic metabolism of thyroid hormones, hormone biosynthesis, transport, intracellular activation and its regulation by the hypothalamic-pituitary axis. Potential interplays between thyroid hormone receptor and PPAR (Hunter *et al*., 1996; Cai *et al*., 1996) will be investigated. If alterations of thyroid hormones are confirmed, the consequences will be investigated. To enhance the interpretation of the experimental data associated with endocrine disruption, additional studies will be conducted to examine neurological and metabolic consequences (two thyroid hormone-associated physiological functions) to correlate and substantiate the findings with circulating hormones.

3. How does this research support the conceptual model for addressing this LTG?

Alterations of thyroid hormones, reported in different animal models after exposure to PFOS, may represent a hallmark MOA of PFAA exposure. The research described here will address provide a better understanding of the unique profile of hormonal changes induced by PFOS. More importantly, an in-depth investigation of functional responses in thyroid hormone-associated target organs will clarify the significance of the unique profile of thyroid hormone alterations.

4. If this project is successful, what products or tools will result from the effort?

They will provide a sound basis for OPPT to use the thyroid hormone changes as an endpoint for risk assessment of PFAA and tther chemicals (such as the conazole pesticides) that have been shown to produce a similar pattern of hormonal responses. Hence, an understanding of the functional significance of these hormonal changes will broadly support the risk assessment of these endocrine disrupting chemicals.

APM. Description of thyroid hormone disruption potentials of PFAA. (RTD/Lau)	**FY09**
APM. Report on thyroid hormone disruption and PFAA: The potential of thyroid hormone action of PFAA to alter brain function (NTD/Gilbert)	**FY10**

Approach H: Development of risk assessment model for PFAA: pharmacodynamic considerations.

1. What is/are the key OPPTS problem(s) that this research effort will address?

Ultimately, OPPT will face the challenge of providing a risk assessment of all the PFAA detected in humans and wildlife, collectively. While this is a long-term issue, a research strategy should be developed to seek viable avenues to attain this lofty goal.

2. What is the proposed research approach?

A potential solution to assess the health risks of PFAA as a class of chemicals is to capitalize common modes of actions for their adverse effects. While the MOA of PFOS (the prototype of PFAA) is not well understood, several possibilities have been suggested that merit further investigation. These include: (a) interference of cholesterol and lipid synthesis and transport (Haughorn and Spydevold, 1992); (b) alterations of mitochondrial bioenergetics (Starkov and Wallace, 2002); (c) peroxisome proliferation (Ikeda *et al*., 1987; Sohlemius *et al*., 1992, 1993; Berhiaume and Wallace, 2002); and (d) inhibition of intercellular communication through gap junction (Hu *et al*., 2002). Although a definitive linkage of these possibilities to overt toxicities has not been established, additional investigation (as such those described in the previous Approaches) will lend credence to these MOA for pharmacodynamic modeling. Currently, the modeling capability described above is not readily available, but can be acquired in the future should sufficient interests and needs be identified. Investigators (postdoctoral fellows, collaborators from other institutions) with specific expertise can be recruited to address various issues described.

3. How does this research support the conceptual model for addressing this LTG?

This research should integrate the various Approaches described in this LTG and provide a conceptual frame work to the risk assessment of the entire class of PFAA.

4. If this project is successful, what products or tools will result from the effort?

Should common MOA be detected among various PFAA, models such as (structure-activity-response, SAR) can be constructed to predict toxicities based on the carbon-chain lengths and functional substitutes of PFAA.

Research Products:

APG Objective: To provide OPPTS a database of toxicity profiles and mechanistic information for various perfluoroalkyl acids (PFAA) in laboratory animal and wildlife models, in order to facilitate the risk assessment of these chemicals.

APM	Title	Contact
FY07	Characterization of PFOA developmental toxicity in the mouse.	Lau (RTD)
FY08	Pharmacokinetic profile of PFOA in the mouse.	Lau (RTD)
FY08	Determine the effects of PFOA exposure on the adult immune system.	Luebke (ETD)
FY08	Report on the critical developmental period for PFOS-induced neonatal mortality in the rat and the effects of PFOS on perinatal development of the lung in the rat.	Rogers (RTD)
FY08	Carcinogenic potentials of PFOA in mouse, possible involvement of endocrine imbalance.	Fenton (RTD)
FY09	Description of thyroid hormone disruption potentials of PFAA.	Lau (RTD)
FY09	Determine the relative sensitivity of the developing immune system to PFAA.	Luebke (ETD)
FY09	Effects of PFAA on developmental expression and activation of PPAR signaling pathways.	Abbott (RTD)
FY09	Determine the PFAA doses required for effects on the developing reproductive systems of male and female rodent offspring	Fenton (RTD)
FY10	Pharmacokinetic models for selected PFAA for interspecies extrapolation.	Lau (RTD)
FY10	Determine the role of PPAR signaling pathway in PFOA-induced immunotoxicity.	Luebke (ETD)
FY10	Identification of the MOA for PFOS and PFOA developmental toxicity by genomic and proteomic approaches.	Rosen (RTD)
FY10	Report on thyroid hormone disruption and PFAA: The potential of thyroid hormone action of PFAA to alter brain function	Gilbert (NTD)
FY11	Evaluation of developmental toxicity of other PFAA.	Lau (RTD)
FY11	Determine effects of PFAA on PPAR signaling functions that impact prenatal and postnatal growth regulation.	Abbott (RTD)
FY11	Report on MOA for tissue dysplasia/tumor formation following early life exposure to PFAA.	Fenton (RTD)

Program Project Area 9: Effects of Novel Herbicides

Approach A. Effects of Novel Herbicides

Project 1 Effects of Novel Herbicides- Spatial Analysis

1. What are the key OPPTS problems this research effort will address?

OPPTS needs regional spatial analysis to identify species for development of testing protocols and to integrate data for risk characterization purposes, especially for novel hazards such as low-dose herbicides.

2. What is the proposed research approach?

Spatial information will be compiled in a GIS platform using ARCINFO (or other GIS software). The GIS platform will be made available at least initially by scientists and risk assessors within the EPA through a web page. If proven successful and feasible, we will make the platform and web page available to the public. The geographic areas identified with the GIS platform as having the greatest risk for non-target herbicide effects will be candidates for more detailed spatial probabilistic analysis of herbicide impacts on a case-by-case basis.

3. How does this research support the conceptual model for addressing this LTG?

This project will improve models and databases to determine ecological risks of novel or newly-discovered potential hazards (especially low-dose herbicides) to plants in a spatially explicit landscape.

4. **If this project is successful, what products or tools will result from the effort?**

This project will provide databases, a web-based GIS platform for use in spatial analysis, and a probabilistic modeling approach to assess the magnitude of the risk from chemical herbicides to non-target crops and native species. These tools also will be applicable to evaluate risks from other classes of pesticides besides herbicides, as well as other chemicals.

APG148	Improved methods to assess direct and indirect risk to nontarget species from use of herbicides.	9	
APM469	Guidelines for regional approach to selection of plant species for herbicide risk assessment	6	WED
APM 63	Refined regional assessment tools for probabilistic assessments of risks to plants from herbicides based on GIS framework	9	WED

Project 2: Effects of Novel Herbicides- Phytoxicity Tests

1. What are the key OPPTS problems this research effort will address?

OPP is mandated under FIFRA to evaluate the potential ecological risks of crop pesticide drift to non-target plants as part of their risk assessment process. Current phytotoxicity tests focus on growth responses of small seedling crop plants, which may not predict effects on reproduction which are important for crop yield and wildlife habitat, or on regionally important crop or native plant species.

2. What is the proposed research approach?

The goal of the research is to improve phytotoxicity (Tier II) testing guidelines. The focus of the research is on terrestrial plant effects with pilot research on aquatic plants. Candidate crop and non-crop test species for plant tests will be based on spatial analysis, prior use in phytotoxicity testing, ecological significance, and cultural characteristics. These may include biennial or perennial crops (herbaceous or woody) and native plants (both annual and perennial). Species will be evaluated to improve traditional seedling (e.g. vegetative vigor) tests and proposed life-cycle (reproductive/developmental) tests. Plants will be grown both in greenhouses and in field plots.

3. How does this research support the conceptual model for addressing this LTG?

This project will produce comprehensive and efficient *in vivo* assays to evaluate adverse effects of novel or newly-discovered potential hazards (especially low-dose herbicides) at critical plant life stages, and will develop new approaches for tier II testing for those chemicals, including methods for native plant species.

4. **If this project is successful, what products or tools will result from the effort?**

This project will provide tests to determine effects of herbicides on crop and native plant species for terrestrial plant risk assessments. The products will be applicable to evaluate risks from other classes of pesticides besides herbicides, as well as other chemicals. Much of the new knowledge gained regarding phytotoxic effects of herbicides on terrestrial plants could be applied to aquatic plant research in the future.

APG 148	Improved methods to assess direct and indirect risk to nontarget species from use of herbicides.	9	
APM 470	Draft guidelines for revised protocol for vegetative vigor test with crops and selected native plants	6	WED
APM 246	Draft protocols for new guidelines for reproductive / developmental endpoints with annual species	8	WED

Project 3: Effects of Novel Herbicides- Ecological Tests

1. What are the key OPPTS problems this research effort will address?

One of the key questions in OPPTS ecological risk assessments is the ability to predict changes in native plants, especially threatened or endangered plant species, in response to novel hazards such as low-dose herbicides. Current phytotoxicity tests need new information to be better applicable to native plants, and data are needed for improved ecological tests (Tier III) which may be required on a case-by-case basis.

2. What is the proposed research approach?

Initially, ecological information will be obtained under controlled greenhouse conditions to determine the relative herbicide susceptibility of different native species based on exposure response studies with individual plants growing in pots. Large field-plot studies will be conducted to refine and further develop methodology for determining herbicide effects at the plant community. Both constructed communities with planted species and *in situ* native plant communities would be studied.

3. How does this research support the conceptual model for addressing this LTG?

This project will produce comprehensive and efficient *in vivo* and potentially *in situ* assays to evaluate ecological risks of novel or newly-discovered potential hazards (especially low-dose herbicides) on native plants. The assays can be used to develop guidelines for improved Tier III ecological tests which may be required on a case-by-case basis.

4. **If this project is successful, what products or tools will result from the effort?**

If successful this project will provide tools to improve ecological risk assessments for herbicide effects on non-target native vegetation. The products will be applicable to evaluate risks from other classes of pesticides besides herbicides as well as other chemicals.

APG 148	Improved methods to assess direct and indirect risk to nontarget species from use of herbicides.	9	
APM 470	Draft guidelines for revised protocol for vegetative vigor test with crops and selected native plants	6	WED
APM 246	Draft protocols for new guidelines for reproductive / developmental endpoints with annual species	8	WED

Project 4: Effects of Novel Herbicides- Chemical/Molecular Tests

1. What is/are the key OPPTS problem(s) that this research effort will address?

OPPTS needs new methods to assess the ecological risks associated with particular pesticides, particularly for chemicals such as low-dose herbicides which do not leave any residue in the plants that could be detected through tissue chemical analysis. New chemical or molecular indicators of potential herbicide effects must be specific for herbicides in question and possibly indicate differences in plant species susceptibility to the herbicide.

2. What is the proposed research approach?

In terms of chemical indicators, our studies initially will focus on 2-aba and will determine the reliability of the metabolite as an indicator of ALS-inhibiting herbicide effects considering herbicide concentration, time of sampling after exposure to the herbicide, and sensitivity of the method to different ALS-inhibiting families of herbicides. For molecular indicators we will evaluate whether gene arrays can be used to indicate whether a plant has been affected by specific herbicides and what the possible effects of the herbicides may be based on the gene activity, and whether this molecular information can in the future be used for quick tests. We will determine if molecular indicators can predict the potential susceptibility of different plant species to an herbicide (especially native plants and/or threatened and endangered plant species). For aquatic plants we will conduct pilot research to determine the feasibility of using photosystem II fluorescence kinetics to measure effects of high potency herbicides.

3. How does this research support the conceptual model for addressing this LTG?

We will produce comprehensive and efficient *in vitro* chemical/molecular assays to evaluate adverse effects of novel or newly-discovered potential hazards (especially low-dose herbicides) to plants. We will develop protocols for chemical/molecular tests to characterize risks to species beyond those initially tested and for herbicides with similar modes-of-activity.

4. **If this project is successful, what products or tools will result from the effort?**

This project will provide genomics- and proteomics-based molecular approaches to assess the magnitude of the risk from chemical herbicides to non-target crops and native species. The approaches could be used to develop rapid tests for herbicide drift to plants in the field. These tools will be applicable to evaluate risks from other classes of pesticides besides herbicides. Knowledge gained regarding chemical/molecular effects of herbicides on terrestrial plants could be applied to aquatic plant research in the future.

APG 148	Improved methods to assess direct and indirect risk to nontarget species from use of herbicides.	9	
APM	Develop molecular methods for tracking exposure and assessing effects of plants exposed to low dose, high potency herbicides	8	WED

Program Project Area 10: Dealing with the emerging risks of new or existing pesticides and other chemicals.

Project 1: Determining the priority for research with newly discovered hazards related to pesticides and chemicals

Introduction:

With the passage of time, increasingly sophisticated technologies are being developed to produce commercial products and pesticides which meet consumer needs but that do not possess the toxic, bioaccumulative and other undesirable properties of the existing chemicals that they were designed to replace. However, despite the best intent on the part of manufacturers, past experience has shown that some are likely to display novel properties that exert unintended adverse effects. Dealing with novel or newly discovered hazards such as chemical pesticides and toxic chemicals can be exceedingly difficult for at least two reasons. First, the fundamental dilemma is that evidence of an emerging hazard must reach some critical threshold before scientists and science managers can agree that it exists - but there is no consensus on what constitutes that threshold. The second, practical, reason is that once there is agreement that a hazard exists, questions inevitably arise regarding what to do about it and what resources can be marshaled.

Criteria:

Long-term Goal 4 exists to provide a mechanism for ORD in support of OPPTS to research these substances providing a scientific basis to better assess their risks and to better understand their novel properties and the significance of these properties with respect to the safe use of the chemicals. Emerging issues that warrant the attention for support under LTG 4 will likely arise over time. Deciding what research to support under LTG 4 requires a consideration of multiple scientific, societal, political and management concerns which vary from issue to issue. Thus, OPPTS and ORD need to establish a formal process to identify and prioritize the work that will be conducted under LTG 4 to decide when to start new research and when to phase out or realign existing research efforts. Given the relatively limited number of research areas that can be supported under this Long-term Goal, we suggest that the RCT, and key partners in OPPTS work together to carefully consider proposed additions to and deletions from the research program and the vet those recommendations though senior leadership of ORD and OPPTS. However, to make the process of deciding what research to conduct transparent and clear, it is important to have criteria and a process that are understood and accepted. Given these considerations, we propose the following guidance for identifying and dealing with emerging hazards.

1. Deciding to Initiate a Research Program Under LTG 4

1) The problem is novel and/or newly discovered in that current methods, models and technologies do not provide an adequate basis to evaluate risk
2) Although little is known about the nature and extent of hazard associated with exposure, there is significant concern about the potential hazards associate with the chemical or class of substances because early indications suggest that the hazards will be important,

if true, and prudence dictates that research should be done to obtain a clearer picture of safety

3) Study of the novel substance(s) will likely yield new understandings about chemical risk that can be applied more broadly to other classes of compounds.
4) Exposure is high and widely spread across the U.S. and/or world
5) The novel substance is EPA's responsibility
6) There is little or no work underway in other agencies, in academia or industry to conduct research on the hazards and/or EPA leadership is needed to ensure that the results of such research will be responsive to EPA's needs.
7) NHEERL has the necessary expertise to address the problems associated with the novel/newly discovered issue
8) NHEERL and OPPTS senior management agree that the research should be supported

2. For Deciding to Phase Out a Research Program Under LTG 4

1) Research has resulted in knowledge such that the problem methods, models and technologies have been generated to provide an adequate basis to evaluate risk and/or to conclude that the problem is no longer novel
2) Knowledge has been generated to demonstrate that the risks associated with exposure to the chemical/class of chemicals is not a significant concern
3) Study of the novel substance(s) has yielded new understandings about chemical risk that can be applied more broadly to other classes of compounds.
4) NHEERL and OPPTS senior management agree that the research no longer should be supported under LTG 4
5) The research has reached a logical conclusion
6) It is no longer possible to secure a budget sufficient to support the work

All appendicies can be found on the NHEERL SP2 Website

http://www.nheerl.epa.gov/nheerl_science/pesticide_implementation/